

09/882,509

(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

L1 42118 S STREPTOKINASE?  
L2 630 S "SKC" OR "SKC-2"  
L3 42539 S L1 OR L2  
L4 7454183 S CLON? OR EXPRESS? OR RECOMBINANT  
L5 4154 S L3 AND L4  
L6 1362 S EQUISIMILIS  
L7 220 S L5 AND L6  
L8 49433 S INCLUSION (W) BOD?  
L9 5 S L7 AND L8  
L10 1 DUP REM L9 (4 DUPLICATES REMOVED)  
L11 106 DUP REM L7 (114 DUPLICATES REMOVED)  
L12 5 S LAMNDA  
L13 1 S L11 AND INCLUSION  
L14 0 S L11 AND AGGREGAT?  
L15 0 S L11 AND INSOLUBLE  
L16 1 S L11 AND SOLUBL?  
L17 71 S L11 AND COLI  
E KUPPUSAMY M/AU  
L18 40 S E3  
E ELLA K/AU  
E KHATRI G S/AU  
L19 46 S E3  
E LAHIRI S/AU  
L20 1660 S E3  
E SRINIVAS V K/AU  
L21 28 S E3  
L22 1842 S L17 OR L18 OR L19 OR L20 OR L21  
L23 71 S L3 AND L22  
L24 1 S L8 AND L23  
L25 71 S L4 AND L23  
L26 71 S L6 AND L25  
L27 1 S L26 AND L8

=>

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spectral property data  
NEWS 13 DEC 05 CASREACT(R) - Over 10 million reactions available  
NEWS 14 DEC 14 2006 MeSH terms loaded in MEDLINE/LMEDLINE  
NEWS 15 DEC 14 2006 MeSH terms loaded for MEDLINE file segment of TOXCENTER  
NEWS 16 DEC 14 CA/Caplus to be enhanced with updated IPC codes  
NEWS 17 DEC 16 MARPATprev will be removed from STN on December 31, 2005  
NEWS 18 DEC 21 IPC search and display fields enhanced in CA/Caplus with the  
IPC reform  
NEWS 19 DEC 23 New IPC8 SEARCH, DISPLAY, and SELECT fields in USPATFULL/USPAT2  
  
NEWS EXPRESS DECEMBER 02 CURRENT VERSION FOR WINDOWS IS V8.01,  
CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),  
AND CURRENT DISCOVER FILE IS DATED 02 DECEMBER 2005.  
V8.0 USERS CAN OBTAIN THE UPGRADE TO V8.01 AT  
<http://download.cas.org/express/v8.0-Discover/>  
  
NEWS HOURS STN Operating Hours Plus Help Desk Availability  
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=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci	SINCE FILE	TOTAL
COST IN U.S. DOLLARS	ENTRY	SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 17:12:17 ON 29 DEC 2005

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FILE 'NTIS' ENTERED AT 17:12:17 ON 29 DEC 2005  
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FILE 'LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005  
COPYRIGHT (C) 2005 Cambridge Scientific Abstracts (CSA)

=> s streptokinase?  
L1 42118 STREPTOKINASE?

=> s "skc" or "SKC-2"  
L2 630 "SKC" OR "SKC-2"

=> s l1 or l2  
L3 42539 L1 OR L2

=> s clon? or express? or recombinant  
L4 7454183 CLON? OR EXPRESS? OR RECOMBINANT

=> s l3 and l4  
L5 4154 L3 AND L4

=> s equisimilis  
L6 1362 EQUISIMILIS

=> s l5 and l6  
L7 220 L5 AND L6

=> s inclusion (w) bod?  
L8 49433 INCLUSION (W) BOD?

=> s l7 and l8  
L9 5 L7 AND L8

=> dup rem l9  
PROCESSING COMPLETED FOR L9

L10 1 DUP REM L9 (4 DUPLICATES REMOVED)

=> d all

L10 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 1  
AN 2000:96119 BIOSIS  
DN PREV200000096119  
TI Two **streptokinase** genes are **expressed** with different  
solubility in *Escherichia coli* W3110.  
AU Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria;  
Fernandez, Julio; Paez, Rolando; Torrens, Isis  
CS Biopharmaceutical Development Division, Center for Genetic Engineering and  
Biotechnology, Havana, Cuba  
SO Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.  
CODEN: BILED3. ISSN: 0141-5492.  
DT Article  
LA English  
ED Entered STN: 15 Mar 2000  
Last Updated on STN: 3 Jan 2002  
AB The **streptokinase** (SK) gene from *S. equisimilis* H46A  
(ATCC 12449) was **cloned** in *E. coli* W3110 under the control of  
the tryptophan promoter. The **recombinant** SK, which represented  
15% of total cell protein content, was found in the soluble fraction of  
disrupted cells. The solubility of this SK notably differed from that of  
the product of the SK gene from *S. equisimilis* (ATCC 9542) which  
had been **cloned** in *E. coli* W3110 by using similar  
**expression** vector and cell growth conditions, and occurred in the  
form of **inclusion bodies**.  
CC Genetics of bacteria and viruses 31500  
Biochemistry methods - Nucleic acids, purines and pyrimidines 10052  
Biochemistry methods - Proteins, peptides and amino acids 10054  
Replication, transcription, translation 10300  
Biophysics - Molecular properties and macromolecules 10506  
Microbiological apparatus, methods and media 32000  
Food microbiology - General and miscellaneous 39008  
Enzymes - General and comparative studies: coenzymes 10802  
Metabolism - Proteins, peptides and amino acids 13012  
Morphology and cytology of bacteria 30500  
Physiology and biochemistry of bacteria 31000  
IT Major Concepts  
Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics  
(Biochemistry and Molecular Biophysics)  
IT Chemicals & Biochemicals  
amino acids; enzymes; proteins; tryptophan  
IT Miscellaneous Descriptors  
biotechnology; cell growth conditions; **expression** vectors;  
gene **expression**; promoters; tryptophan promoter  
ORGN Classifier  
Enterobacteriaceae 06702  
Super Taxa  
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria;  
Microorganisms  
Organism Name  
*Escherichia coli*: W 3110  
Taxa Notes  
Bacteria, Eubacteria, Microorganisms  
ORGN Classifier  
Gram-Positive Cocci 07700  
Super Taxa  
Eubacteria; Bacteria; Microorganisms  
Organism Name  
*Streptococcus equisimilis*  
Taxa Notes



Bacteria, Eubacteria, Microorganisms  
RN 54-12-6Q (tryptophan)  
73-22-3Q (tryptophan)

=> d his

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L7 220 S L5 AND L6  
L8 49433 S INCLUSION (W) BOD?  
L9 5 S L7 AND L8  
L10 1 DUP REM L9 (4 DUPLICATES REMOVED)

=> dup rem l7

PROCESSING COMPLETED FOR L7

L11 106 DUP REM L7 (114 DUPLICATES REMOVED)

=> s lamnda

L12 5 LAMNDA

=> d 1-106 ibib ab

L12 ANSWER 1 OF 5 MEDLINE on STN  
ACCESSION NUMBER: 77076903 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 794695  
TITLE: A ribosomal RNA gene of Escherichia coli (rrnD) on  
lamnda daro E specialized transducing phages.  
AUTHOR: Jorgensen P  
SOURCE: Molecular & general genetics : MGG, (1976 Aug 2) 146 (3)  
303-7.  
Journal code: 0125036. ISSN: 0026-8925.  
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 197702  
ENTRY DATE: Entered STN: 19900313  
Last Updated on STN: 19900313  
Entered Medline: 19770216

AB Lambda-transducing phages carrying segments of the Escherichia coli  
chromosome in the aroE-trkA region have been isolated and shown by  
hybridization to carry an rRNA gene (rrnD). The most likely gene order is  
trkA aroE rrnD. The EcoRI and SmaI endonuclease cutting pattern of the  
rrnD gene is identical with the one of rrnB, differentiated from rrnC.

L12 ANSWER 2 OF 5 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights  
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ACCESSION NUMBER: 78013410 EMBASE  
DOCUMENT NUMBER: 1978013410  
TITLE: Coli phages found in human feces as free virions.  
AUTHOR: Kawabata N.; Hirakawa K.; Mukai H.; Nakamura K.  
CORPORATE SOURCE: Dept. Bacteriol., Fac. Med., Kagoshima Univ., Kagoshima,  
Japan  
SOURCE: Acta Medica Universitatis Kagoshimaensis, (1976) Vol. 18,  
No. 2, pp. 103-112.

CODEN: AMUKAC  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 047 Virology  
004 Microbiology  
LANGUAGE: English

AB The present studies provided information on the density and properties of coliphages which existed as free virions in freshly excreted human feces. The phages active (propagating) on coli strains B and C and certain shigella strains were concerned. Marked differences were observed in phage content among individual fecal samples. Three of 9 samples contained coli phages in numbers sufficient to be detected by the plating method. The density was of the order of  $10^3$  to  $10^4$  PFU/ml irrespective of the host strains used. Coli cells in the same samples counting  $10^5$  to  $10^6$ /ml, the phage/cell ratios ranged between  $10^{-2}$  and  $10^{-3}$ . Seventy four phages were isolated and examined. They were classified into more than 5 types on the basis of their morphological and serological features. Forty one phages evidenced a morphological appearance characteristic to T even coli phages, and 26 among them were related serologically as well to T even phages. So it follows that true and related T even forms were widespread and predominant in human intestinal tract as free virions. There were also obtained 6 phages which resembled morphologically E1 phage of Bradley (1963) but were heterogenous in serological features, and, furthermore, 3 phages which were morphologically comparable to the lambda phage but serologically distinct from it. The lambda type phages, though propagated on E. coli C, lysed none of the coli isolates from fecal samples.

L12 ANSWER 3 OF 5 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1994:622437 SCISEARCH  
THE GENUINE ARTICLE: NF684  
TITLE: M-FUNCTION BEHAVIOR FOR A PERIODIC DIRAC SYSTEM  
AUTHOR: CLEMENCE D P (Reprint)  
CORPORATE SOURCE: UNIV ZIMBABWE, DEPT MATH, POB MP 167, HARARE, ZIMBABWE (Reprint)  
COUNTRY OF AUTHOR: ZIMBABWE  
SOURCE: PROCEEDINGS OF THE ROYAL SOCIETY OF EDINBURGH SECTION A-MATHEMATICS, (1994) Vol. 124, Part 1, pp. 149-159.  
ISSN: 0308-2105.  
PUBLISHER: ROYAL SOC EDINBURGH, 22-24 GEORGE ST, EDINBURGH, MIDLOTHIAN, SCOTLAND EH2 2PQ.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: PHYS  
LANGUAGE: English  
REFERENCE COUNT: 3  
ENTRY DATE: Entered STN: 1994  
Last Updated on STN: 1994

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB For a  $2 \times 2$  periodic system with a perturbation  $P$  whose first moment is finite,  $Jy' = [\lambda I + R(x) + P(x)]y$ , we study the behaviour of the Titchmarsh-Weyl  $m(\lambda)$ -coefficient at the spectral gap endpoints. Assuming gap nondegeneracy, our main results is that as  $\lambda \rightarrow \lambda_0$ ,  $(\lambda - \lambda_0)^{1/2} \rightarrow$  not-equal 0 if and only if  $\lambda_0$  is a  $\phi$ -half-bound state, which follows from an analysis of Jost-type functions.

L12 ANSWER 4 OF 5 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1991:655190 SCISEARCH  
THE GENUINE ARTICLE: GR307  
TITLE: PROJECTING PRECIPITOUSNESS  
AUTHOR: GOLDRING N (Reprint)  
CORPORATE SOURCE: UNIV CALIF LOS ANGELES, DEPT MATH, LOS ANGELES, CA 90024 (Reprint)

COUNTRY OF AUTHOR: USA  
SOURCE: ISRAEL JOURNAL OF MATHEMATICS, (1991) Vol. 74, No. 1, pp. 13-31.  
ISSN: 0021-2172.  
PUBLISHER: MAGNES PRESS, PO BOX 7695, JERUSALEM 91076, ISRAEL.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: PHYS  
LANGUAGE: English  
REFERENCE COUNT: 10  
ENTRY DATE: Entered STN: 1994  
Last Updated on STN: 1994

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB This paper is about "strong" ideals on small cardinals. It is shown that a typical property of large cardinal measures does not transfer to these ideals. More specifically, that precipitous ideals on  $P$ -omega-1-lambda spaces may not project down to precipitous ideals on "smaller"  $P$ -omega-1-lambda spaces. Also, that the existence of a presaturated ideal on the bigger space does not imply the existence of a presaturated ideal on the smaller space.

L12 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 2005:1161439 HCAPLUS  
DOCUMENT NUMBER: 143:412054  
TITLE: Measurement of Lambda(c) branching fractions of Cabibbo-suppressed decay modes in the BABAR experiment  
AUTHOR(S): Saleem, M.  
CORPORATE SOURCE: State Univ. of New York, Albany, NY, USA  
SOURCE: (2005) 144 pp. Avail.: UMI, Order No. DA3164608  
From: Diss. Abstr. Int., B 2005, 66(2); 961  
DOCUMENT TYPE: Dissertation  
LANGUAGE: English  
AB Unavailable

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L9 5 S L7 AND L8  
L10 1 DUP REM L9 (4 DUPLICATES REMOVED)  
L11 106 DUP REM L7 (114 DUPLICATES REMOVED)  
L12 5 S LAMNDA

=> d l11 1-106 ibib ab

L11 ANSWER 1 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN  
ACCESSION NUMBER: 2005441717 EMBASE  
TITLE: Heberkinasa: Recombinant streptokinase [9].  
AUTHOR: Hernandez L.; Martinez Y.; Quintana M.; Besada V.; Martinez E.  
CORPORATE SOURCE: L. Hernandez, Production Division, Centro de Ingenieria Genetica Y Biotecnologia, Ave 31 e/ 158 y 190, Cubanacan,

SOURCE: Playa, Habana 0600, Cuba. luciano.hernandez@cigb.edu.cu  
 European Heart Journal, (2005) Vol. 26, No. 16, pp. 1691.  
 Refs: 4  
 ISSN: 0195-668X CODEN: EHJODF  
 COUNTRY: United Kingdom  
 DOCUMENT TYPE: Journal; Letter  
 FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery  
 030 Pharmacology  
 037 Drug Literature Index  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 20051020  
 Last Updated on STN: 20051020

L11 ANSWER 2 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:55585 SCISEARCH  
 THE GENUINE ARTICLE: 758JR  
 TITLE: Acquisition of host plasmin activity by the swine pathogen  
 Streptococcus suis serotype 2  
 AUTHOR: Jobin M C; Brassard J; Quessy S; Gottschalk M; Grenier D  
 (Reprint)  
 CORPORATE SOURCE: Univ Laval, Grp Rech Ecol Buccale, Fac Med Dent, Quebec  
 City, PQ G1K 7P4, Canada (Reprint); Univ Montreal, Fac Med  
 Vet, Grp Rech Malad Infect Porc, St Hyacinthe, PQ J2S 7C6,  
 Canada; Univ Montreal, Fac Med Vet, Canadian Res Network  
 Bacterial Pathogens Swine, Nat Sci & Engr Res Council  
 Canada, St Hyacinthe, PQ J2S 7C6, Canada  
 COUNTRY OF AUTHOR: Canada  
 SOURCE: INFECTION AND IMMUNITY, (JAN 2004) Vol. 72, No. 1, pp.  
 606-610.  
 ISSN: 0019-9567.  
 PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC  
 20036-2904 USA.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 28  
 ENTRY DATE: Entered STN: 23 Jan 2004  
 Last Updated on STN: 23 Jan 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In this study, the plasminogen-binding activity of Streptococcus suis  
 serotype 2 was investigated. Bound human plasminogen was activated by  
 purified streptokinase, urokinase, or Streptococcus dysgalactiae  
 subsp. equisimilis culture supernatant. Both human and porcine  
 plasminogen were bound by S. suis. Binding was inhibited by  
 E-aminocaproic acid, and the plasminogen receptor was heat and sodium  
 dodecyl sulfate resistant. One of the receptors was identified as  
 glyceraldehyde-3-phosphate dehydrogenase. S. suis-associated plasmin  
 activity was capable of activating free plasminogen, which in turn could  
 contribute to degradation of fibronectin. This is the first report on the  
 plasminogen-binding activity of S. suis. Further studies may reveal a  
 contribution of this activity to the virulence of S. suis.

L11 ANSWER 3 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:897332 HCAPLUS  
 DOCUMENT NUMBER: 142:190437  
 TITLE: Development of a green fluorescent protein  
 metastatic-cancer chick-embryo drug-screen model  
 AUTHOR(S): Bobek, Vladimir; Plachy, Jiri; Pinterova, Daniela;  
 Kolostova, Katarina; Boubelik, Michael; Jiang, Ping;  
 Yang, Meng; Hoffman, Robert M.  
 CORPORATE SOURCE: Department of Molecular Biology, Third Faculty of  
 Medicine Charles University Prague, Prague, Czech Rep.  
 SOURCE: Clinical & Experimental Metastasis (2004), 21(4),  
 347-352

CODEN: CEXMD2; ISSN: 0262-0898

PUBLISHER: Kluwer Academic Publishers  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The chick-embryo model has been an important tool to study tumor growth, metastasis, and angiogenesis. However, an imageable model with a genetic fluorescent tag in the growing and spreading cancer cells that is stable over time has not been developed. We report here the development of such an imageable fluorescent chick-embryo metastatic cancer model with the use of green fluorescent protein (GFP). Lewis lung carcinoma cells, stably expressing GFP, were injected on the 12th day of incubation in the chick embryo. GFP-Lewis lung carcinoma metastases were visualized by fluorescence, after seven days addnl. incubation, in the brain, heart, and sternum of the developing chick embryo, with the most frequent site being the brain. The combination of **streptokinase** and gemcitabine was evaluated in this GFP metastatic model. Twelve-day-old chick embryos were injected i.v. with GFP-Lewis lung cancer cells, along with these two agents either alone or in combination. The **streptokinase** -gemcitabine combination inhibited metastases at all sites. The ED of gemcitabine was found to be 10 mg/kg and **streptokinase** 2000 IU per embryo. The data in this report suggest that this new stably fluorescent imageable metastatic-cancer chick-embryo model will enable rapid screening of new antimetastatic agents. Abbreviation: GFP - green fluorescent protein.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 4 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:69727 SCISEARCH

THE GENUINE ARTICLE: 763KK

TITLE: **Streptokinase** - a clinically useful thrombolytic agent

AUTHOR: Banerjee A; Chisti Y; Banerjee U C (Reprint)

CORPORATE SOURCE: Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Sector 67, Mohali 160062, Punjab, India (Reprint); Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Mohali 160062, Punjab, India; Massey Univ, Inst Technol & Engn, Palmerston North, New Zealand

COUNTRY OF AUTHOR: India; New Zealand

SOURCE: BIOTECHNOLOGY ADVANCES, (FEB 2004) Vol. 22, No. 4, pp. 287-307.  
ISSN: 0734-9750.

PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.

DOCUMENT TYPE: General Review; Journal

LANGUAGE: English

REFERENCE COUNT: 171

ENTRY DATE: Entered STN: 30 Jan 2004  
Last Updated on STN: 30 Jan 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A failure of hemostasis and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infraction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and **streptokinase**. This review deals with **streptokinase** as a clinically important and cost-effective plasminogen activator. The aspects discussed include: the mode of action; the structure and structure-function relationships; the structural modifications for improving functionality; **recombinant streptokinase**; microbial production; and recovery of this protein from crude broths. (C) 2003 Published by Elsevier Inc.

L11 ANSWER 5 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on

STN

ACCESSION NUMBER: 2004:656928 SCISEARCH  
THE GENUINE ARTICLE: 837ZE  
TITLE: Genetic analysis of Streptococcus uberis plasminogen activators  
AUTHOR: Ward P N (Reprint); Leigh J A  
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7HN, Berks, England (Reprint); Inst Anim Hlth, Compton Lab, Compton RG20 7NN, Berks, England  
phil.ward@bbscr.ac.uk  
COUNTRY OF AUTHOR: England  
SOURCE: INDIAN JOURNAL OF MEDICAL RESEARCH, (MAY 2004) Vol. 119, Supp. [S], pp. 136-140.  
ISSN: 0971-5916.  
PUBLISHER: INDIAN COUNCIL MEDICAL RES, PO BOX 4911 ANSARI NAGAR, NEW DELHI 110029, INDIA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 14  
ENTRY DATE: Entered STN: 13 Aug 2004  
Last Updated on STN: 13 Aug 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Background & objectives: Streptococci produce a diverse range of secreted plasminogen activators capable of converting mammalian plasminogen to plasmin in a species-specific manner. In all examples to date, the host animal's plasminogen and that of a number of additional species have been shown to interact with these molecules leading to the conclusion that the pathogenesis of streptococci is in some way dependent upon activation of host plasminogen. PauA was the first plasminogen activator described from Streptococcus uberis, a pathogen frequently isolated from cases of bovine mastitis. Recently, a second S. uberis plasminogen activator (PauB) was identified from a Danish mastitis isolate. Interestingly, the pauB open reading frame occupied the locus normally filled by pauA. In the present study a genetic screen of streptococcal and field isolates frequently associated with mastitis was undertaken to assess the distribution, chromosomal location and sequence variation of these putative virulence factors.

Methods: Southern analysis of a diverse panel of streptococci and additional bacterial isolates frequently associated with bovine mastitis was performed using pauA and pauB probes. Sequence variation of PauA was assessed at the protein level following nucleotide sequence analysis of pauA alleles amplified from isolates picked from different geographical locations.

Results: We observed plasminogen activators to be universally distributed amongst S. uberis. A pauA allele was identified in all but one strain of S. uberis. This strain had a pauB allele substituted for pauA at the same locus. The remarkably low level of sequence variation demonstrated by PauA was further restricted to a limited number of residues within the molecule.

Interpretation & conclusion: The high prevalence of PauA alleles in, field isolates of S. uberis supported the observation that plasminogen activators are likely to confer an advantage with respect to colonization and growth. The findings of the present study support the theory that PauA plays a critical role in the pathogenesis of S. uberis.

L11 ANSWER 6 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1

ACCESSION NUMBER: 2004:656911 SCISEARCH  
THE GENUINE ARTICLE: 837ZE  
TITLE: Control of streptokinase gene expression in group A & C streptococci by two-component regulators  
AUTHOR: Malke H (Reprint); Steiner K  
CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena,

Germany  
hmalke@imb-jena.de  
COUNTRY OF AUTHOR: Germany  
SOURCE: INDIAN JOURNAL OF MEDICAL RESEARCH, (MAY 2004) Vol. 119,  
Supp. [S], pp. 48-56.  
ISSN: 0971-5916.  
PUBLISHER: INDIAN COUNCIL MEDICAL RES, PO BOX 4911 ANSARI NAGAR, NEW  
DELHI 110029, INDIA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 23  
ENTRY DATE: Entered STN: 13 Aug 2004  
Last Updated on STN: 13 Aug 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Background & objectives: Group A streptococci (GAS) and human isolates of group C streptococci (GCS) have the stable capacity to produce the plasminogen activator **streptokinase**, albeit with varying efficiency. This property is subject to control by two two-component regulatory systems, FasCAX and CovRS, which act as activator and repressor, respectively. The present work aims at balancing these opposing activities in GAS and GCS, and at clarifying the phylogenetic position of the FasA response regulator, the less understood regulator of the two systems.

Methods: The GCS strain H46A and GAS strain NZ131 were used. *Escherichia coli* JM 109 was used as host for plasmid construction. **Streptokinase** activity of various wild type and mutant strains was measured. Phylogenetic trees of streptococcal FasA homologues were established.

Results: The **streptokinase** activities of the GAS strain NZ131 and the GCS strain H46A were attributable to more efficient CovR repressor action in NZ131 than in H46A. The FasA activator, on the other hand, functioned about equally efficient in the two strains. Phylogenetically, FasA homologues clustered distinctly in the proposed FasA-BlpR-CovE family of streptococcal response regulators and used the LytTR domain for DNA binding.

Interpretation & conclusion: Assessing the apparent **streptokinase** activity of streptococcal strains require the dissection of the activities of the cov and fas systems. Although experimental evidence is still missing, FasA is closely related to a widely distributed family of streptococcal response regulators that is involved in behavioral processes, such as quorum sensing.

L11 ANSWER 7 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN DUPLICATE 2

ACCESSION NUMBER: 2003404343 EMBASE

TITLE: Expression of streptodornase by use of **streptokinase** promoter in *Streptococcus equisimilis* H46A.

AUTHOR: Sohn H.-J.; Chin J.; Kim I.-C.; Bai S.; Lee H.B.

CORPORATE SOURCE: H.B. Lee, Department of Biological Sciences, Chonnam National University, Gwangju 500-757, Korea, Republic of. blaise@chonnam.chonnam.ac.kr

SOURCE: Korean Journal of Microbiology and Biotechnology, (2003) Vol. 31, No. 3, pp. 307-310.  
Refs: 18

ISSN: 1598-642X CODEN: HMHAAS

COUNTRY: Korea, Republic of

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: Korean

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 20031023

Last Updated on STN: 20031023

AB A gene encoding streptodornase(sdc) from *Streptococcus equisimilis*

H46A was expressed in *S. equisimilis* H46A sdc(-) under the control of the **streptokinase** gene promoter. Secretion of the streptodornase was directed by the signal sequences of **streptokinase** or streptodornase. The expressed streptodornase activity from *S. equisimilis* H46A sdc(-) transformant with **streptokinase** promoter - streptodornase coding sequence fusion vector was 2.3 fold higher than that from wild type. Construct of signal sequence region replaced by **streptokinase** ones was similarly expressed as a wild type. But constructs of **skc** or **lrp** core regions of **streptokinase** promoter streptodornase fusion were similarly expressed as in sdc(-) mutant. In conclusion, improved expression of streptodornase by use of **streptokinase** promoter required the full length of promoter.

L11 ANSWER 8 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN DUPLICATE 3

ACCESSION NUMBER: 2003:133401 BIOSIS  
DOCUMENT NUMBER: PREV200300133401  
TITLE: Structural correlates of a functional **streptokinase** antigenic epitope: Serine 138 is an essential residue for antibody binding.  
AUTHOR(S): Parhami-Seren, Behnaz [Reprint Author]; Seavey, Matthew; Krudysz, Jolanta; Tsantili, Panayota  
CORPORATE SOURCE: Department of Biochemistry, College of Medicine, University of Vermont, 89 Beaumont Street, Given Building, Burlington, VT, 05405-0068, USA  
bparhami@zoo.uvm.edu  
SOURCE: Journal of Immunological Methods, (15 January 2003) Vol. 272, No. 1-2, pp. 93-105. print.  
ISSN: 0022-1759 (ISSN print).  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 12 Mar 2003  
Last Updated on STN: 12 Mar 2003

AB We determined the pattern of cross-reactivity of a panel of anti-**streptokinase** (SK) monoclonal antibodies (mAbs) with SK variants in order to map the antigenic and functional epitope of SK. Comparison of the pattern of cross-reactivity of the anti-SK mAb A4.3 with SK variants and sequence alignments of SK variants and native (n) SK suggested that mutation of Ser 138 to Lys results in loss of binding of mAb A4.3 to SK variants. However, this mutation does not affect formation of activator complex by these proteins. The epitope specificity of the mAb A4.3 was further confirmed by mutating Ser 138 to Lys in n SK. Monoclonal Ab A4.3 did not bind to mutant SK (Ser138Lys). Activator activity of mutant SK (Ser138Lys) was indistinguishable from that of n SK and recombinant n SK. Since addition of A4.3 mAb to an equimolar mixture of SK and human plasminogen inhibits activator complex formation, the sequences spanning position 138 are likely important for formation of **streptokinase**-plasminogen activator complex or processing of the plasminogen substrate.

L11 ANSWER 9 OF 106 MEDLINE on STN DUPLICATE 4  
ACCESSION NUMBER: 2002322701 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12065504  
TITLE: Dual control of **streptokinase** and streptolysin S production by the covRS and fasCAX two-component regulators in *Streptococcus dysgalactiae* subsp. *equisimilis*.  
AUTHOR: Steiner Kerstin; Malke Horst  
CORPORATE SOURCE: Institute for Molecular Biology, Friedrich Schiller University Jena, D-07745 Jena, Germany.  
SOURCE: Infection and immunity, (2002 Jul) 70 (7) 3627-36.  
Journal code: 0246127. ISSN: 0019-9567.  
PUB. COUNTRY: United States



DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AY075106; GENBANK-AY075107  
ENTRY MONTH: 200207  
ENTRY DATE: Entered STN: 20020615  
Last Updated on STN: 20020731  
Entered Medline: 20020730

AB Synthesis of the plasminogen activator **streptokinase** (SK) by group A streptococci (GAS) has recently been shown to be subject to control by two two-component regulators, covRS (or csrRS) and fasBCA. In independent studies, response regulator CovR proved to act as the repressor, whereas FasA was found to act indirectly as the activator by controlling the **expression** of a stimulatory RNA, fasX. In an attempt at understanding the regulation of SK production in the human group C streptococcal (GCS) strain H46A, the strongest SK producer known yet, we provide here physical and functional evidence for the presence of the cov and fas systems in GCS as well and, using a mutational approach, compare the balance between their opposing actions in H46A and GAS strain NZ131. Sequence analysis combined with Southern hybridization revealed that the covRS and fasCAX operons are preserved at high levels of primary structure identity between the corresponding GAS and GCS genes, with the exception of fasB, encoding a second sensor kinase that is not a member of the GCS fas operon. This analysis also showed that wild-type H46A is actually a derepressed mutant for SK and streptolysin S (SLS) synthesis, carrying a K102 amber mutation in covR. Using cov and fas mutations in various combinations together with strain constructs allowing complementation in trans, we found that, in H46A, cov and fas contribute to approximately equal negative and positive extents, respectively, to constitutive SK and SLS activity. The amounts of SK paralleled the level of skc(H46A) transcription. The most profound difference between H46A and NZ131 regarding the relative activities of the cov and fas systems consisted in significantly higher activity of a functional CovR repressor in NZ131 than in H46A. In NZ131, CovR decreased SK activity in a Fas(+) background about sevenfold, compared to a 1.9-fold reduction of SK activity in H46A. Combined with the very short-lived nature of covR mRNA (decay rate, 1.39/min), such differences may contribute to strain-specific peculiarities of the **expression** of two prominent streptococcal virulence factors in response to environmental changes.

L11 ANSWER 10 OF 106 MEDLINE on STN DUPLICATE 5  
ACCESSION NUMBER: 2002053807 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 11779212  
TITLE: Specificity role of the **streptokinase** C-terminal domain in plasminogen activation.  
AUTHOR: Kim Dong Min; Lee Sang Jun; Yoon Suk Kwon; Byun Si Myung  
CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 305-701 Taejeon, South Korea.  
SOURCE: Biochemical and biophysical research communications, (2002 Jan 11) 290 (1) 585-8.  
Journal code: 0372516. ISSN: 0006-291X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200202  
ENTRY DATE: Entered STN: 20020125  
Last Updated on STN: 20020226  
Entered Medline: 20020225

AB Several pathogenic bacteria secrete plasminogen activator proteins. **Streptokinase** (SKe) produced by *Streptococcus equisimilis* and staphylokinase secreted from *Staphylococcus aureus* are human

plasminogen activators and **streptokinase** (SKu), produced by *Streptococcus uberis*, is a bovine plasminogen activator. Thus, the fusion proteins among these activators can explain the function of each domain of SKe. Replacement of the SKalpha domain with staphylokinase donated the staphylokinase-like activation activity to SKe, and the SKbetagamma domain played a role of nonproteolytic activation of plasminogen.

**Recombinant** SKu also activated human plasminogen by staphylokinase-like activation mode. Because SKu has homology with SKe, the bovine plasminogen activation activities of SKe fragments were checked. SKebetagamma among them had activation activity with bovine plasminogen. This means that the C-terminal domain (gamma-domain) of **streptokinase** determines plasminogen species necessary for activation and converses the ability of substrate recognition to human species.

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ACCESSION NUMBER: 2002110457 EMBASE  
TITLE: Can imperfections help to improve bioreactor performance?.  
AUTHOR: Patnaik P.R.  
CORPORATE SOURCE: P.R. Patnaik, Institute of Microbial Technology, Sector 39-A, Chandigarh-160 036, India. pratap@imtech.res.in  
SOURCE: Trends in Biotechnology, (1 Apr 2002) Vol. 20, No. 4, pp. 135-137.  
Refs: 22  
ISSN: 0167-7799 CODEN: TRBIDM  
PUBLISHER IDENT.: S 0167-7799(01)01922-9  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; General Review  
FILE SEGMENT: 004 Microbiology  
027 Biophysics, Bioengineering and Medical Instrumentation  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 20020404  
Last Updated on STN: 20020404

AB Pilot-scale and larger bioreactors differ from small laboratory-scale reactors in terms of a greater occurrence of noise and incomplete mixing of the broth. Conventional control tries to induce good mixing and to filter out the noise as completely as possible. As such an 'ideal' operation is difficult to achieve, recent work has tried to exploit the non-ideal features to improve the performance. Using artificial neural networks, the degree of mixing, the extent of filtering of noise and the distribution of plasmid copy number (in a **recombinant** fermentation) can be controlled effectively on-line. This strategy generates better productivities than well-mixed noise-free operations, which suggests that deviations from ideal behaviour should be gainfully harnessed and not suppressed.

L11 ANSWER 12 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:21007 SCISEARCH  
THE GENUINE ARTICLE: 503LT  
TITLE: Characterization of PauB, a novel broad-spectrum plasminogen activator from *Streptococcus uberis*  
AUTHOR: Ward P N; Leigh J A (Reprint)  
CORPORATE SOURCE: Compton Lab, Inst Anim Hlth, Compton RG20 7NN, Berks, England (Reprint)  
COUNTRY OF AUTHOR: England  
SOURCE: JOURNAL OF BACTERIOLOGY, (JAN 2002) Vol. 184, No. 1, pp. 119-125.  
ISSN: 0021-9193.  
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC

20036-2904 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 35  
ENTRY DATE: Entered STN: 11 Jan 2002  
Last Updated on STN: 11 Jan 2002

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A bovine plasminogen activator of atypical molecular mass (similar to 45 kDa) from Streptococcus uberis strain SK880 had been identified previously (L. B. Johnsen, K. Poulsen, M. Kilian, and T. E. Petersen. Infect. Immun. 67:1072-1078, 1999). The strain was isolated from a clinical case of bovine mastitis. The isolate was found not to secrete PauA, a bovine plasminogen activator **expressed** by the majority of S. uberis strains. Analysis of the locus normally occupied by pauA revealed an absence of the pauA open reading frame. However, an alternative open reading frame was identified within the same locus. Sequence analysis of the putative gene suggested limited but significant homology to other plasminogen activators. A candidate signal peptide sequence and cleavage site were also identified. **Expression** cloning of DNA encoding the predicted mature protein (lacking signal peptide) confirmed that the open reading frame encoded a plasminogen activator of the expected size, which we have named PauB. Both native and **recombinant** forms of PauB displayed an unexpectedly broad specificity profile for bovine, ovine, equine, caprine, porcine, rabbit, and human plasminogen. Clinical and nonclinical field isolates from nine United Kingdom sites were screened for the pauB gene and none were identified as carrying it. Similarly, clinical isolates from 20 Danish herds were all found to encode PauA and not PauB. Therefore, PauB represents a novel but rare bacterial plasminogen activator which displays very broad specificity.

L11 ANSWER 13 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:471828 HCAPLUS  
DOCUMENT NUMBER: 133:99568  
TITLE: **Streptokinase** derivatives with high affinity for activated platelets and methods of their production and use in thrombolytic therapy  
INVENTOR(S): Galler, Lawrence Isaac  
PATENT ASSIGNEE(S): USA  
SOURCE: U.S., 15 pp.  
CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6087332	A	20000711	US 1997-997532	19971223
PRIORITY APPLN. INFO.:			US 1997-997532	19971223

OTHER SOURCE(S): MARPAT 133:99568

AB **Streptokinase** derivs. having platelet glycoprotein-binding domains adjacent to the termini of the **streptokinase** sequence are disclosed. These derivs. produce higher local concns. of plasmin in vivo as compared to unmodified **streptokinase**. Certain of the derivs. have high affinity for the GPIIB/IIIA receptor and low affinity for the fibronectin and vitronectin receptors. Others have substantially equivalent affinity for all three receptors. The derivs. are useful in treating thromboembolic disorders. The **streptokinase** derivs. can be made by **recombinant** techniques or by chemical synthesis or conjugation.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 14 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:29440 HCAPLUS

DOCUMENT NUMBER: 142:428913

TITLE: An improved process for the simultaneous preparation of extracellular streptokinase and its analogues

INVENTOR(S): Dikshit, Kanak Lata; Vyas, Vinay Venkatrao; Mahajan, Ritu; Kaur, Jaodeep; Thapar, Nitika; Phatap, Jitesh; Nihalani, Deepak; Sahni, Girish

PATENT ASSIGNEE(S): Council of Scientific and Industrial Research, India  
SOURCE: Indian, 122 pp.

CODEN: INXXAP

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IN 183828	A	20000429	IN 1994-DE1727	19941230
PRIORITY APPLN. INFO.:			IN 1994-DE1727	19941230

AB Extracellular streptokinase and its analogs prepared by growing recombinant E.coli in a conventional fermentation medium under stirring and supplemented with aeration, separating the cells from supernatant by known methods followed by recovering and purifying Streptokinase and its analogs from supernatant.

L11 ANSWER 15 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:175570 HCAPLUS

DOCUMENT NUMBER: 132:218864

TITLE: Streptokinase analogs with low antigenicity for use as thrombolytics

INVENTOR(S): Torrens Madrazo, Isis Del Carmen; Garcia Ojalvo, Ariana; De La Fuente Garcia, Jose De Jesus; Seralena Menendez, Alina

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba

SOURCE: Eur. Pat. Appl., 54 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 985729	A2	20000315	EP 1999-202639	19990813
EP 985729	A3	20000531		
EP 985729	B1	20050427		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9943424	A1	20000309	AU 1999-43424	19990805
AU 769916	B2	20040212		
CA 2277554	C	20041102	CA 1999-2277554	19990806
CA 2277554	AA	20000214		
US 6309873	B1	20011030	US 1999-374038	19990813
AT 294239	E	20050515	AT 1999-202639	19990813
US 6413759	B1	20020702	US 2000-658179	20000908
PRIORITY APPLN. INFO.:			CU 1998-119	A 19980814
			US 1999-374038	A3 19990813

AB Streptokinase analogs with antigenic domains modified to minimize antigenicity are described for use in the treatment of clotting-associated disorders. The proteins retain their capacity for plasminogen activator complex formation. The proteins are manufactured by

expression of the corresponding allele of the skc2 gene encoding streptokinase SKC-2 (Heberkinase®). The mols. obtained from present invention can be used in the treatment of disorders as myocardial infarction, pulmonary thromboembolism, surgical complications and other cases of thrombosis.

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ACCESSION NUMBER: 2001003323 EMBASE  
TITLE: Leucine 42 in the fibronectin motif of streptokinase plays a critical role in fibrin-independent plasminogen activation.  
AUTHOR: Liu L.; Sazonova I.Y.; Turner R.B.; Chowdhry S.A.; Tsai J.; Hounq A.K.; Reed G.L.  
CORPORATE SOURCE: G.L. Reed, Cardiovascular Biology Laboratory, HSPH II-127, 677 Huntington Ave., Boston, MA 02115, United States. reed@cvtlab.harvard.edu  
SOURCE: Journal of Biological Chemistry, (1 Dec 2000) Vol. 275, No. 48, pp. 37686-37691.  
Refs: 35  
ISSN: 0021-9258 CODEN: JBCHA3  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 20010111  
Last Updated on STN: 20010111

AB The NH(2) terminus (residues 1-59) of streptokinase (SK) is a molecular switch that permits fibrin-independent plasminogen activation. Targeted mutations were made in recombinant (r) SK1-59 to identify structural interactions required for this process. Mutagenesis established the functional roles of Phe-37 and Glu-39, which were projected to interact with microplasmin in the activator complex. Mutation of Leu-42 (rSK1-59(L42A)), a conserved residue in the SK fibronectin motif that lacks interactions with microplasmin, strongly reduced plasminogen activation (k(cat) decreased 50-fold) but not arnidolysis (k(cat) decreased 1.5-fold). Otherwise rSK1-59(L42A) and native rSK1-59 were indistinguishable in several parameters. Both displayed saturable and specific binding to Glu-plasminogen or the remaining SK fragment (rSKA59). Similarly rSK1-59 and rSK1-59(L42A) bound simultaneously to two different plasminogen molecules, indicating that both plasminogen binding sites were intact. However, when bound to SKA59, rSK1-59(L42A) was less effective than rSK1-59 in restructuring the native conformation of the SK A domain, as detected by conformation-dependent monoclonal antibodies. In the light of previous studies, these data provide evidence that SK1-59 contributes to fibrin-independent plasminogen activation through 1) intermolecular inter-actions with the plasmin in the activator complex, 2) binding interactions with the plasminogen substrate, and 3) intramolecular interactions that structure the A domain of SK for Pg substrate processing.

L11 ANSWER 17 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:148580 SCISEARCH  
THE GENUINE ARTICLE: 285UW  
TITLE: Allele substitution of the streptokinase gene reduces the nephritogenic capacity of group A streptococcal strain NZ131  
AUTHOR: Nordstrand A (Reprint); McShan W M; Ferretti J J; Holm S E; Norgren M  
CORPORATE SOURCE: Umea Univ, Dept Clin Bacteriol, S-90185 Umea, Sweden (Reprint); Univ Oklahoma, Hlth Sci Ctr, Dept Microbiol & Immunol, Oklahoma City, OK 73190 USA

COUNTRY OF AUTHOR: Sweden; USA  
SOURCE: INFECTION AND IMMUNITY, (MAR 2000) Vol. 68, No. 3, pp. 1019-1025.  
ISSN: 0019-9567.  
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 36  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB To investigate the role of allelic variants of **streptokinase** in the pathogenesis of acute poststreptococcal glomerulonephritis (APSGN), site-specific integration plasmids were constructed, which contained either the non-nephritis-associated **streptokinase** gene (*skc5*) from the group C streptococcal strain *Streptococcus equisimilis* H46A or the nephritis-associated **streptokinase** gene (*skal*) from the group A streptococcal nephritogenic strain NZ131. The plasmids were introduced by electroporation and homologous recombination into the chromosome of an isogenic derivative of strain NZ131, in which the **streptokinase** gene had been deleted and which had thereby lost its nephritogenic capacity in a mouse model of APSGN. The introduction of a non-nephritis-associated allelic variant of **streptokinase** did not rescue the nephritogenic capacity of the strain. The mutant and the wild-type strains produced equivalent amounts of **streptokinase**. Complementation of the *ska* deletion derivative with the original *ska* allele reconstituted the nephritogenicity of wild-type NZ131. The findings support the hypothesis that the role of **streptokinase** in the pathogenesis of APSGN is related to the allelic variant of the protein.

L11 ANSWER 18 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:100732 SCISEARCH  
THE GENUINE ARTICLE: 277JH  
TITLE: Genetic organisation of the M protein region in human isolates of group C and G streptococci: two types of multigene regulator-like (*mgrC*) regions  
AUTHOR: Geyer A; Schmidt K H (Reprint)  
CORPORATE SOURCE: Univ Jena, Univ Hosp, Inst Med Microbiol, Semmelweisstr 4, D-07740 Jena, Germany (Reprint); Univ Jena, Univ Hosp, Inst Med Microbiol, D-07740 Jena, Germany  
COUNTRY OF AUTHOR: Germany  
SOURCE: MOLECULAR AND GENERAL GENETICS, (JAN 2000) Vol. 262, No. 6, pp. 965-976.  
ISSN: 0026-8925.  
PUBLISHER: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 47  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In addition to beta-haemolytic streptococci belonging to Lancefield group A (*Streptococcus pyogenes*, GAS), human isolates of group C (GCS) and group G (GGS) streptococci (*S. dysgalactiae* subsp. *equisimilis*;) have been implicated as causative agents in outbreaks of purulent pharyngitis, of wound infections and recently also of streptococcal toxic shock-like syndrome. Very little is known about the organisation of the genomic region in which the *emm* gene of GCS and GCS is located. We have investigated the genome sequences flanking the *emm* gene in GCS by sequencing neighbouring fragments obtained by inverse PCR. Our sequence data for GCS strains 25287 and H46A revealed two types of arrangement in

the emm region, which differ significantly from the known types of mga regulon in GAS. We named this segment of the genome mgrC (for multigene regulon-like segment in group C streptococci). In strains belonging to the first mgrC type (prototype strain 25287) the emm gene is flanked upstream by mgc, a gene that is 61% identical to the mga gene of GAS. A phylogenetic analysis of the deduced protein sequences showed that Mgc is related to Mga proteins of various types of GAS but forms a distinct cluster. Downstream of emm, the mgrC sequence region is bordered by rel. This gene encodes a protein that functions in the synthesis and degradation of guanosine 3',5' bipyrophosphate (ppGpp) during the stringent regulatory response to amino acid deprivation. In the second mgrC type (prototype strain H46A), the genes mgc and emm are arranged as in type 1. But an additional ORF (orf) is inserted in opposite orientation between emm and rel. This orf shows sequence homology to cpdB, which is present in various microorganisms and encodes 2',3' cyclo-nucleotide 2'-phosphodiesterase. PCR analysis showed that these two mgrC arrangements also exist in GGS. Our sequence and PCR data further showed that both types of mgrC region in GCS and GGS are linked via rel to the streptokinase region characterised recently in strain H46A. A gene encoding C5a peptidase, which is present at the 3' end of the mga regulon in GAS, was not found in the mgrC region identified in the GCS and GGS strains investigated here.

L11 ANSWER 19 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:126480 HCAPLUS  
DOCUMENT NUMBER: 133:218185  
TITLE: Firefly luciferase as a reporter to study gene expression in Streptococcus mutans. [Erratum to document cited in CA131:347148]  
AUTHOR(S): Goodman, Steven D.; Gao, Qian  
CORPORATE SOURCE: Department of Basic Sciences, University of Southern California School of Dentistry, Los Angeles, CA, 90089-0641, USA  
SOURCE: Plasmid (2000), 43(2), 184  
CODEN: PLSMDX; ISSN: 0147-619X  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The firefly (*Photinus pyralis*) luciferase coding sequence has previously been used as a reporter in the genus *Streptococcus* [S. Grafe, T. Ellinger, and H. Malke (1996) Structural dissection and functional anal. of the complex promoter of the streptokinase gene from *Streptococcus equisimilis* H46A. Med. Microbiol. Immunol. 185, 11-17]. The text on page 154, second paragraph, lines 13 and 14 should be amended to read "...it has been underutilized in gram-pos. hosts.". (c) 2000 Academic Press.

L11 ANSWER 20 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 6

ACCESSION NUMBER: 2000:387699 BIOSIS  
DOCUMENT NUMBER: PREV200000387699  
TITLE: Expression and regulation of the streptokinase gene.  
AUTHOR(S): Malke, Horst [Reprint author]; Steiner, Kerstin; Gase, Klaus; Frank, Carsten  
CORPORATE SOURCE: Institute for Molecular Biology, Friedrich Schiller University Jena, D-07745, Jena, Germany  
SOURCE: Methods (Orlando), (June, 2000) Vol. 21, No. 2, pp. 111-124. print.  
CODEN: MTHDE9. ISSN: 1046-2023.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 13 Sep 2000  
Last Updated on STN: 8 Jan 2002

AB Recent research in various areas has appreciably expanded our knowledge of **streptokinase**, a plasminogen activator produced by all human group A (GAS), group C (GCS), and group G (GGS) streptococci. Several molecular genetic approaches are described here to study the **expression** of the **streptokinase** gene, **skn**. Southern hybridization analysis demonstrated homology of synteny of **ska**, **skc**, and **skg** in the genomes of the above serogroups. S1 nuclease mapping, the use of transcriptional fusions to BETA-galactosidase and luciferase reporter genes, in conjunction with site-directed mutagenesis, led to the localization of the core promoter region of **skc** and the identification of a **cis**-active upstream region required for full promoter activity. Circular permutation analysis of the promoter upstream region identified an intrinsic DNA bending locus as the pivotal DNA element stimulating the activity of the core promoter. The detection of **skn** allele-specific **expression** phenotypes, which proved not to be due to different **skn** mRNA half-lives, prompted allele swap experiments, showing that promoter activity is dictated by the host genetic background, rather than the sequence of the regulatory region. These findings suggest the involvement in **skn** **expression** of an as yet unidentified transcriptional activator that contacts the bent DNA region. Transcription termination of **skc** is directed by a bidirectional terminator whose structural requirements for termination efficiency were determined with base substitution mutants fused to a chloramphenicol acetyl transferase reporter. Finally, mutagenic plasmids are described for insertion-duplication and allele replacement mutagenesis of the **skn** locus.

L11 ANSWER 21 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:405093 HCAPLUS  
DOCUMENT NUMBER: 131:54027  
TITLE: Fibrin-dependent plasminogen activator activity of modified bacterial **streptokinases**  
INVENTOR(S): Reed, Guy L.  
PATENT ASSIGNEE(S): The President and Fellows of Harvard College, USA  
SOURCE: PCT Int. Appl., 73 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9931247	A1	19990624	WO 1998-US26694	19981215
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 9918295	A1	19990705	AU 1999-18295	19981215
US 6210667	B1	20010403	US 1998-211542	19981215
PRIORITY APPLN. INFO.:			US 1997-69497P	P 19971215
			WO 1998-US26694	W 19981215

AB A pharmaceutical composition in a preferred embodiment comprises an isolated bacterial protein **streptokinase** that induces fibrin-dependent plasminogen activation, and methods for dissolving blood clots in a subject using such a composition. Two preferred **streptokinase** mutants and truncated derivs. comprising residues 144-293 and residues 60-414 of the *Streptococcus equisimilis* H46A enzyme. Deletion of the first 59 amino acids to product mutant rSK60-414 yielded a protein with a 767-fold decrease in kcat compared to that of rSK1-414, without any



significant change in the Km. The N-terminus dets. the clot (fibrin) dependence of plasminogen activation by **streptokinase** and the regulation of plasminogen activation in the presence of fibrin. By virtue of its requirement for fibrin for plasminogen activation in human plasma, and its sparing of fibrinogen during clot dissoln., **streptokinase** deleted of N-terminal amino acid residues is similar to tissue-type plasminogen activator. Embodiments also include a nucleic acid encoding such as a bacterial protein, a nucleic acid encoding such a bacterial protein as a fusion to another protein, an **expression** vector with the nucleic acid, and a host cell transformed with the **expression** vector.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 22 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:579938 HCAPLUS

DOCUMENT NUMBER: 131:183948

TITLE: Manufacture of **streptokinase** for therapeutic use by **expression** of the cloned gene in *Pichia pastoris*

INVENTOR(S): Estrada Garcia, Mario; Rubiera Chaplen, Roger; Perez, Hidalgo; Serrano Doce, Ricardo; Hernandez Marrero, Luciano F.; Rodriguez Collazo, Pedro; Castro Ramirez, Anaisel; Munoz Munoz, Emilio Amable; Bravo Martinez, Walfrido; Campos Somavilla, Magalys; Pedraza Fernandez, Alicia; De la Furente Garcia, Jose de J.; Herrera Martinez, Luis S.

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba

SOURCE: Czech Rep., 18 pp.

CODEN: CZXXED

DOCUMENT TYPE: Patent

LANGUAGE: Czech

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CZ 284692	B6	19990217	CZ 1991-2256	19910719
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A 19900523
			CS 1991-2256	A 19910719
			SU 1991-5001280	A 19910717

AB **Streptokinase** for therapeutic use is manufactured by **expression** of the cloned gene in *Pichia pastoris*. The protein may be secreted into the culture medium or accumulated intracellularly. The gene was cloned from a type C *Streptococcus equisimilis* by PCR. **Expression** of the gene from the AOX1 promoter using *Pichia pastoris* as the host resulted in the manufacture of an enzyme with a specific activity of 50,000-100,000 fibrin-agarose units/mg protein.

L11 ANSWER 23 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:88244 BIOSIS

DOCUMENT NUMBER: PREV200000088244

TITLE: Hydrodynamic radii of native and denatured proteins measured by pulse field gradient NMR techniques.

AUTHOR(S): Wilkins, Deborah K.; Grimshaw, Shaun B.; Receveur, Veronique; Dobson, Christopher M.; Jones, Jonathan A.; Smith, Lorna J. [Reprint author]

CORPORATE SOURCE: Oxford Centre for Molecular Sciences, New Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QT, UK

SOURCE: Biochemistry, (Dec. 14, 1999) Vol. 38, No. 50, pp.  
16424-16431. print.  
CODEN: BICHAW. ISSN: 0006-2960.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 10 Mar 2000  
Last Updated on STN: 3 Jan 2002

AB Pulse field gradient NMR methods have been used to determine the effective hydrodynamic radii of a range of native and nonnative protein conformations. From these experimental data, empirical relationships between the measured hydrodynamic radius (Rh) and the number of residues in the polypeptide chain (N) have been established; for native folded proteins  $R_h = 4.75N^{0.29}$  Å and for highly denatured states  $R_h = 2.21N^{0.57}$  Å. Predictions from these equations agree well with experimental data from dynamic light scattering and small-angle X-ray or neutron scattering studies reported in the literature for proteins ranging in size from 58 to 760 amino acid residues. The predicted values of the hydrodynamic radii provide a framework that can be used to analyze the conformational properties of a range of nonnative states of proteins. Several examples are given here to illustrate this approach including data for partially structured molten globule states and for proteins that are unfolded but biologically active under physiological conditions. These reveal evidence for significant coupling between local and global features of the conformational ensembles adopted in such states. In particular, the effective dimensions of the polypeptide chain are found to depend significantly on the level of persistence of regions of secondary structure or features such as hydrophobic clusters within a conformational ensemble.

L11 ANSWER 24 OF 106 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 2000038313 MEDLINE

DOCUMENT NUMBER: PubMed ID: 10569766

TITLE: Cloning, expression, sequence analysis,  
and characterization of streptokinases secreted  
by porcine and equine isolates of *Streptococcus*  
*equisimilis*.

AUTHOR: Caballero A R; Lottenberg R; Johnston K H

CORPORATE SOURCE: Department of Microbiology, Immunology and Parasitology,  
Louisiana State University Medical Center, New Orleans,  
Louisiana 70112, USA.

CONTRACT NUMBER: R01DK45014 (NIDDK)

SOURCE: Infection and immunity, (1999 Dec) 67 (12) 6478-86.  
Journal code: 0246127. ISSN: 0019-9567.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AF104300; GENBANK-AF104301

ENTRY MONTH: 199912

ENTRY DATE: Entered STN: 20000113

Last Updated on STN: 20000113

Entered Medline: 19991220

AB Streptokinases secreted by nonhuman isolates of group C streptococci (*Streptococcus equi*, *S. equisimilis*, and *S. zooepidemicus*) have been shown to bind to different mammalian plasminogens but exhibit preferential plasminogen activity. The streptokinase genes from *S. equisimilis* strains which activated either equine or porcine plasminogen were cloned, sequenced, and expressed in *Escherichia coli*. The streptokinase secreted by the equine isolate had little similarity to any known streptokinases secreted by either human or porcine isolates. The streptokinase secreted by the porcine isolate had limited structural and functional similarities to streptokinases secreted by human isolates. Plasminogen activation studies with

immobilized (His) (6)-tagged recombinant streptokinases indicated that these recombinant streptokinases interacted with plasminogen in a manner similar to that observed when streptokinase and plasminogen interact in the fluid phase. Analysis of the cleavage products of the streptokinase-plasminogen interaction indicated that human, equine, and porcine plasminogens were all cleaved at the same highly conserved site. The site at which streptokinase was cleaved to form altered streptokinase (Sk\*) was also determined. This study confirmed not only the presence of streptokinases in nonhuman *S. equisimilis* isolates but also that these proteins belong to a family of plasminogen activators more diverse than previously thought.

L11 ANSWER 25 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 8

ACCESSION NUMBER: 2000:96119 BIOSIS  
DOCUMENT NUMBER: PREV200000096119  
TITLE: Two streptokinase genes are expressed with different solubility in *Escherichia coli* W3110.  
AUTHOR(S): Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis  
CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba  
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.  
CODEN: BILED3. ISSN: 0141-5492.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 15 Mar 2000  
Last Updated on STN: 3 Jan 2002

AB The streptokinase (SK) gene from *S. equisimilis* H46A (ATCC 12449) was cloned in *E. coli* W3110 under the control of the tryptophan promoter. The recombinant SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from *S. equisimilis* (ATCC 9542) which had been cloned in *E. coli* W3110 by using similar expression vector and cell growth conditions, and occurred in the form of inclusion bodies.

L11 ANSWER 26 OF 106 MEDLINE on STN DUPLICATE 9

ACCESSION NUMBER: 1999150235 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10024545  
TITLE: Purification and cloning of a streptokinase from *Streptococcus uberis*.  
AUTHOR: Johnsen L B; Poulsen K; Kilian M; Petersen T E  
CORPORATE SOURCE: Protein Chemistry Laboratory, Department of Molecular and Structural Biology, University of Aarhus, DK-8000 Aarhus C, Denmark.  
SOURCE: Infection and immunity, (1999 Mar) 67 (3) 1072-8.  
Journal code: 0246127. ISSN: 0019-9567.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AJ131604; GENBANK-AJ131605; GENBANK-AJ131631  
ENTRY MONTH: 199903  
ENTRY DATE: Entered STN: 19990326  
Last Updated on STN: 19990326  
Entered Medline: 19990312

AB A bovine plasminogen activator was purified from the culture supernatant of the bovine pathogen *Streptococcus uberis* NCTC 3858. After the final reverse-phase high-performance liquid chromatography step a single protein

with a molecular mass of 32 kDa was detected in the active fraction. A partial peptide map was established, and degenerate primers were designed and used for amplification of fragments of the gene encoding the activator. Inverse PCR was subsequently used for obtaining the full-length gene. The *S. uberis* plasminogen activator gene (*skc*) encodes a protein consisting of 286 amino acids including a signal peptide of 25 amino acids. In an amino acid sequence comparison the cloned activator showed an identity of approximately 26% to the streptokinases isolated from *Streptococcus equisimilis* and *Streptococcus pyogenes*. Interestingly, the activator from *S. uberis* was found to lack the C-terminal domain possessed by the streptokinase from *S. equisimilis*. This is apparently a general feature of the streptokinases of this species; biochemical and genetic analysis of 10 additional strains of *S. uberis* revealed that 9 of these were highly similar to strain NCTC 3858. Sequencing of the *skc* gene from three of these strains indicated that the amino acid sequence of the protein is highly conserved within the species.

L11 ANSWER 27 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1999-03949 BIOTECHDS

TITLE: Purification and cloning of a streptokinase  
from *Streptococcus uberis*;  
cattle plasminogen-activator purification and  
characterization

AUTHOR: Johnson L B; Poulsen K; Kilian M; \*Petersen T E

CORPORATE SOURCE: Univ.Aarhus

LOCATION: Protein Chemistry Laboratory, Gustav Wieds Vej 10C, DK-8000  
Aarhus C, Denmark.

Email: tep@mbio.aau.dk

SOURCE: Infect.Immun.; (1999) 67, 3, 1072-78

CODEN: INFIBR

ISSN: 0019-9567

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A cattle plasminogen-activator was purified from the culture supernatant of *Streptococcus uberis* NTCTC 3858. After the final reverse-phase HPLC step, a single protein with a mol.weight of 32,000 was detected in the active fraction. A partial peptide map was established, and degenerate DNA primers were designed and used for amplification of fragments of the gene encoding the activator. Inverse polymerase chain reaction was used for obtaining the full-length gene. The *S. uberis* plasminogen-activator gene (*skc*) encodes a protein consisting of 286 amino acids including a signal peptide of 25 amino acids. In a protein sequence comparison, the cloned activator showed an identity of approximately 26% to the streptokinases isolated from *Streptococcus equisimilis* and *Streptococcus pyogenes*. The activator from *S. uberis* lacked the C-terminal domain possessed by the streptokinase from *S. equisimilis*. This is apparently a general feature of the streptokinases of this species. Sequencing of the *skc* gene from 3 of these strains indicated that the protein sequence of the protein is highly conserved within the species. (32 ref)

L11 ANSWER 28 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation  
on STN

ACCESSION NUMBER: 1999:666643 SCISEARCH

THE GENUINE ARTICLE: 231GM

TITLE: PauA: a novel plasminogen activator from *Streptococcus uberis*

AUTHOR: Rosey E L; Lincoln R A; Ward P N; Yancey R J; Leigh J A  
(Reprint)

CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks,  
England (Reprint); Pfizer Inc, Div Cent Res, Anim Hlth

COUNTRY OF AUTHOR: Biol Discovery, Groton, CT 06340 USA  
 SOURCE: England; USA  
 FEMS MICROBIOLOGY LETTERS, (1 SEP 1999) Vol. 178, No. 1,  
 pp. 27-33.  
 ISSN: 0378-1097.  
 PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
 NETHERLANDS.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 20  
 ENTRY DATE: Entered STN: 1999  
 Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Chromosomal DNA from two geographically distinct isolates of  
 Streptococcus uberis was used to clone the plasminogen activator  
 in an active form in Escherichia coli. The cloned fragments  
 from each strain contained four potential open reading frames (ORFs).  
 That for the plasminogen activator encoded a protein of 286 amino acids  
 (33.4 kDa) which is cleaved between residues 25 and 26 during secretion by  
 S. uberis. The amino acid sequence of the mature protein showed only weak  
 homology (23.5-28%) to streptokinase. The plasminogen activator  
 gene, pauA, in S. uberis was located between two ORFs with high homology  
 to the DNA mismatch repair genes, hexA and hexB, and not on a DNA fragment  
 between the genes encoding an ATP binding cassette transporter protein  
 (abc) and a protein involved in the formation and degradation of guanosine  
 polyphosphates (rel) as is the case for streptokinase in other  
 streptococci. (C) 1999 Federation of European Microbiological Societies.  
 Published by Elsevier Science B.V. All rights reserved.

L11 ANSWER 29 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1998:789174 HCAPLUS  
 DOCUMENT NUMBER: 130:24116  
 TITLE: Production of non-immunogenic proteins by removal of  
 T-cell and B-cell epitopes  
 INVENTOR(S): Carr, Francis Joseph  
 PATENT ASSIGNEE(S): Biovation Ltd., UK  
 SOURCE: PCT Int. Appl., 77 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9852976	A1	19981126	WO 1998-GB1473	19980521
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2290485	AA	19981126	CA 1998-2290485	19980521
AU 9875393	A1	19981211	AU 1998-75393	19980521
AU 736549	B2	20010802		
GB 2339430	A1	20000126	GB 1999-25632	19980521
EP 983303	A1	20000308	EP 1998-922932	19980521
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002512624	T2	20020423	JP 1998-550129	19980521
US 2003153043	A1	20030814	US 2002-300215	20021120
PRIORITY APPLN. INFO.:			GB 1997-10480	A 19970521

GB 1997-16197	A 19970731
GB 1997-25270	A 19971128
US 1997-67235P	P 19971202
GB 1998-7751	A 19980414
WO 1998-GB1473	W 19980521
US 1999-438136	B1 19991110

AB Proteins, or parts of proteins, may be rendered non-immunogenic, or less immunogenic, to a given species by identifying in their amino acid sequences one or more potential epitopes for T-cells of that species. Once identified, these amino acid sequence are modified to eliminate one or more MHC class II-restricted T-cell epitopes. In similar fashion, B-cell epitopes may be removed if desirable. By this process the immunogenicity of the protein when exposed to the immune system of the given species is reduced or eliminated. Monoclonal antibodies and other Ig-like mols. can particularly benefit from being rendered less immunogenic (e.g., humanized antibodies for therapy).

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 30 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 10

ACCESSION NUMBER: 1998:317596 BIOSIS  
 DOCUMENT NUMBER: PREV199800317596  
 TITLE: Effect of signal peptide changes on the extracellular processing of streptokinase from Escherichia coli: Requirement for secondary structure at the cleavage junction.  
 AUTHOR(S): Pratap, J.; Dikshit, K. L. [Reprint author]  
 CORPORATE SOURCE: Inst. Microbial Technology, Sector 39-A, Chandigarh 160 036, India  
 SOURCE: Molecular and General Genetics, (May, 1998) Vol. 258, No. 4, pp. 326-333. print.  
 CODEN: MGGEAE. ISSN: 0026-8925.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 22 Jul 1998  
 Last Updated on STN: 10 Sep 1998

AB Streptokinase (SK), an extracellular protein from Streptococcus equisimilis, is secreted post-translationally by Escherichia coli using both its native and E. coli-derived transport signals. In this communication we report that cleavage specificity of signal peptidase I, and thus efficiency of secretion, varies in E. coli when SK export is directed by different transport signals. The native (+ 1) N-terminus of mature SK was retained when it was transported under the control of its own, PelB or LamB signal peptide. However, when translocation of SK was controlled by the OmpA or MalE signal peptide, Ala2 of mature SK was preferred as a cleavage site for the pre-SK processing. Our results indicate that compatibility of the leader peptide with the mature sequences of SK, which fulfils the requirement for a given secondary structure within the cleavage region, is essential for maintaining the correct processing of pre-SK. An OmpA-SK fusion, which results in the deletion of two N-terminal amino acid residues of mature SK, was further studied with respect to the recognition of alternative cleavage site in E. coli. The alanine at +2 in mature SK was changed to glycine or its relative position was changed to +3 by introducing a methionine residue at the +1 position. Both alterations resulted in the correct cleavage of pre-SK at the original OmpA fusion site. In contrast, introduction of an additional alanine at +4, creating three probable cleavage sites (Ala-x-Ala-x-Ala-x-Ala), resulted in the recognition of all three target sites for cleavage, with varying efficiency. The results indicate that the nature of the secondary structure generated at the cleavage junction of pre-SK, resulting from the fusion of different signal peptides, modulates the cleavage specificity of signal peptidase I during extracellular processing of SK. Based on these findings it is proposed

that flexibility in the interaction of the active site of signal peptidase I with the cleavage sites of signal peptides may occur when it encounters two or more juxtaposed cleavage sites. Preference for one cleavage site over another, then, may depend on fulfillment of secondary structure requirements in the vicinity of the pre-protein cleavage junction.

L11 ANSWER 31 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation  
on STN

ACCESSION NUMBER: 1999:317401 SCISEARCH

THE GENUINE ARTICLE: 189BZ

TITLE: Hydrophobic interaction chromatography applied to  
purification of **recombinant streptokinase**

AUTHOR: Perez N (Reprint); Urrutia E; Camino J; Orta D R; Torres  
Y; Martinez Y; Cruz M; Alburquerque S; Gil M R; Hernandez  
L

CORPORATE SOURCE: Ctr Genet Engn & Biotechnol, Streptokinase Div, Havana,  
Cuba; Ctr Genet Engn & Biotechnol, Qual Control Div,  
Havana, Cuba

COUNTRY OF AUTHOR: Cuba

SOURCE: MINERVA BIOTECNOLOGICA, (DEC 1998) Vol. 10, No. 4, pp.  
174-177.

ISSN: 1120-4826.

PUBLISHER: EDIZIONI MINERVA MEDICA, CORSO BRAMANTE 83-85 INT JOURNALS  
DEPT., 10126 TURIN, ITALY.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 22

ENTRY DATE: Entered STN: 1999

Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Background **Recombinant streptokinase** (rSk) is a  
streptococcal protein cloned in *E. coli*. (11) Several methods  
have been described for **streptokinase** purification: ion exchange  
chromatography, (12) affinity chromatography with canine plasmin (13) and  
chromatography on immobilized acylated human plasminogen. (14) Monoclonal  
antibodies anti-rSk immobilized to Sepharose (15) have been used too.  
Recently this protein was purified using HIC.

Methods. rSk (CIGB, Cuba) was produced by fermentation strain K12 of *E. coli*, (11) the protein was recovered after washed pellet, cellular  
disruption and solubilization. Several purification assays were done  
using TSK-Butyl (Tosohaas, Japan) as a support for hydrophobic interaction  
chromatography (HIC). The protein was loaded to the column with 1 M of  
ammonium sulfate before being washed using an elution gradient from 0.5 to  
0 M of ammonium sulfate, in order to determine the elution point of the  
rSk.

Results. We could determine that this protein is partly hydrophobic,  
this determination was shown by analysis of its aminoacidic sequence.  
This protein has 415 aminoacids of which 36% are non polar. The  
absorption capacity for TSK Butyl 650 S varies from 15 to 20 mg/mL. The  
optimum elution point was obtained using 0.25 M of ammonium sulfate, the  
eluted material was obtained with a high level of purity (<1% of  
contaminants). The recovery of rSk was about 49% using the mean of five  
assays.

Conclusions. The experimental process evaluated could be efficiently  
inserted in a downstream process to obtain **recombinant streptokinase**  
highly purified as final preparation and good  
recovery.

L11 ANSWER 32 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation  
on STN

ACCESSION NUMBER: 1998:473042 SCISEARCH

THE GENUINE ARTICLE: ZV077

TITLE: The interaction of *Streptococcus dysgalactiae* with plasmin

and plasminogen  
 AUTHOR: Leigh J A (Reprint); Hodgkinson S M; Lincoln R A  
 CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks,  
 England (Reprint)  
 COUNTRY OF AUTHOR: England  
 SOURCE: VETERINARY MICROBIOLOGY, (15 MAR 1998) Vol. 61, No. 1-2,  
 pp. 121-135.  
 ISSN: 0378-1135.  
 PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
 NETHERLANDS.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 29  
 ENTRY DATE: Entered STN: 1998  
 Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The activation of plasminogen and the binding of plasmin by bacteria may have many effects which promote infection. The occurrence of such activities in streptococci is well documented; however, these are yet to be demonstrated for *S. dysgalactiae*. Consequently, the ability of this bacterium to activate mammalian plasminogen and bind either plasmin or its zymogen was investigated. Activation of bovine plasminogen was dependant on both the strain and the growth medium used for cultivation. Eighteen strains were able to activate bovine and ovine plasminogen and some of these also activated plasminogen from the horse, rabbit and pig. None activated human plasminogen and one strain (CE127) did not activate plasminogen from any source. Tricine-SDS PAGE and zymographic analysis of culture supernatants showed that bovine plasminogen was activated by four out of six strains at two locations corresponding to 16 kDa and 10 kDa. Following the growth of five strains in the presence of bovine plasminogen, all but strain CE127 bound high levels of plasmin activity. In contrast, following growth in human plasminogen none of the strains exhibited bound plasmin activity although all could bind human plasmin directly. All strains were also able to bind bovine and human plasminogen in such a way as to allow its activation by urokinase. We conclude that *S. dysgalactiae* is capable of activating mammalian plasminogen in a species-specific fashion and that the bacterium is also capable of binding plasmin and plasminogen with an apparent preference for bovine plasmin over human plasmin and/or plasminogen from either species. (C) 1998 Elsevier Science B.V.

L11 ANSWER 33 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
 ACCESSION NUMBER: 1998-11139 BIOTECHDS

TITLE: Streptokinase-mediated plasminogen activation:  
 molecular studies using genetically engineered  
 streptokinase variants;  
 recombinant streptokinase production  
 via plasmid pGEX-3X expression in *Streptococcus*  
*pyogenes* and *Streptococcus equisimilis*

AUTHOR: Lizano S  
 CORPORATE SOURCE: Univ.Costa-Rica  
 LOCATION: Instituto Clodomiro Picardo, Facultad de Microbiologia, and  
 Departamento de Bioquimica, Escuela de Medicina, Universidad  
 de Costa Rica, San Jose, Costa Rica.  
 SOURCE: Biotechnol.Apl.; (1998) 15, 2, 110  
 CODEN: 2048M  
 ISSN: 0864-4551  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB Streptokinase triggers a non-proteolytic activation of plasminogen by forming a stoichiometric complex with the plasminogen. *Streptococcus pyogenes* NZ131, SP13013 and *Streptococcus equisimilis* H46A strains were chosen for this study. After isolation of the streptokinase their sequences were amplified



using polymerase chain reaction. The amplification was followed by ligation into plasmid pUC18 and then subcloning into **expression** vector plasmid pGEX-3X in translational frame with the glutathione-transferase (EC-2.5.1.18) gene (GST). The cells were then transformed and colonies which **expressed** GST-**streptokinase** were selected. An internal polymorphic region of the enzyme molecule (implicated in pathogenesis) was deleted and replaced with a linker. Site-directed mutagenesis was performed and human Glu-plasminogen was purified and the **recombinant** constructs were assayed. This method provided an alternative method to preproteolysis plasminogen activation. Future efforts to characterize the structure/function relationship of **streptokinase** may influence its engineering and so improve its therapeutic potential and explain its role in disease. (7 ref)

L11 ANSWER 34 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:437021 SCISEARCH

THE GENUINE ARTICLE: ZU066

TITLE: The Streptococcus agalactiae hylB gene encoding hyaluronate lyase: completion of the sequence and **expression** analysis

AUTHOR: Gase K; Ozegowski J; Malke H (Reprint)

CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany; Univ Jena, Inst Expt Microbiol, D-07745 Jena, Germany

COUNTRY OF AUTHOR: Germany

SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND EXPRESSION, (29 MAY 1998) Vol. 1398, No. 1, pp. 86-98. ISSN: 0167-4781.

PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 43

ENTRY DATE: Entered STN: 1998

Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We report the **cloning**, sequencing and **expression** analysis of the Streptococcus agalactiae strain 4755 hylB(4755) allele, the first chromosomally-encoded streptococcal hyaluronate lyase gene to be **cloned** and sequenced completely. This gene lies in a region homologous to that found in S. mutans, between the mutX and rmlB genes, a region involved in the synthesis of the serotype c-specific polysaccharide antigen of this organism. Sequencing of hylB(4755) revealed a 3216-bp open reading frame that encodes a 121.2-kDa polypeptide possessing a 30-amino acid signal sequence which was theoretically predicted and experimentally confirmed. A **recombinant** plasmid, pHYB100, containing hylB(4755) together with its promoter and terminator was constructed and used to analyze the **expression** of the gene in Escherichia coli. In Northern hybridization experiments, hylB(4755) was found to be transcribed as 3.3-kb monocistronic mRNA from its own promoter which exhibits an extended, sigma(70)-like 10 consensus sequence. Transcript mapping by primer extension analysis placed the major transcription initiation site leading to the longest transcript 38 bp upstream of the translational initiation codon: ATG. E. coli TG1(pHYB100) efficiently synthesized hyaluronan-cleaving enzyme activity at similar to 7000 working units/10(9) cells, with lyase activity detectable in all principle cellular locations. Zymography and Western analysis identified functional activity in TG1(pHYB100) to be associated with similar to 118, 110 and 94-kDa polypeptides, with the two low molecular weight species constituting the major components of the enzyme purified from the culture supernatant fluid of S. agalactiae 4755. The 118-kDa form was shown to

represent the undegraded mature enzyme, whereas the smaller species are likely to arise from proteolytic cleavage in the N-terminal part of the mature protein. The HylB(4755) protein showed extensive sequence identity to the homologous enzymes from *S. agalactiae* 3502 and *S. pneumoniae* characterized by others but sequence comparisons clearly show that incomplete genes truncated at their 5' ends had been isolated from these two organisms. (C) 1998 Elsevier Science B.V. All rights reserved.

L11 ANSWER 35 OF 106 MEDLINE on STN DUPLICATE 11  
 ACCESSION NUMBER: 1998350778 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 9686161  
 TITLE: Cloning, expression and purification of recombinant streptokinase: partial characterization of the protein expressed in *Escherichia coli*.  
 AUTHOR: Avilan L; Yarzabal A; Jurgensen C; Bastidas M; Cruz J; Puig J  
 CORPORATE SOURCE: Laboratorio de Biologia y Medicina Experimental, Facultad de Ciencias, Universidad de Los Andes, Merida, Venezuela.  
 SOURCE: Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.], (1997 Dec) 30 (12) 1427-30.  
 Journal code: 8112917. ISSN: 0100-879X.  
 PUB. COUNTRY: Brazil  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199810  
 ENTRY DATE: Entered STN: 19981020  
 Last Updated on STN: 19981020  
 Entered Medline: 19981005

AB We cloned the streptokinase (STK) gene of *Streptococcus equisimilis* in an expression vector of *Escherichia coli* to overexpress the profibrinolytic protein under the control of a tac promoter. Almost all the recombinant STK was exported to the periplasmic space and recovered after gentle lysozyme digestion of induced cells. The periplasmic fraction was chromatographed on DEAE Sepharose followed by chromatography on phenyl-agarose. Active proteins eluted between 4.5 and 0% ammonium sulfate, when a linear gradient was applied. Three major STK derivatives of 47.5 kDa, 45 kDa and 32 kDa were detected by Western blot analysis with a polyclonal antibody. The 32-kDa protein formed a complex with human plasminogen but did not exhibit Glu-plasminogen activator activity, as revealed by a zymographic assay, whereas the 45-kDa protein showed a  $K(m) = 0.70 \text{ microM}$  and  $kcat = 0.82 \text{ s}^{-1}$ , when assayed with a chromogen-coupled substrate. These results suggest that these proteins are putative fragments of STK, possibly derived from partial degradation during the export pathway or the purification steps. The 47.5-kDa band corresponded to the native STK, as revealed by peptide sequencing.

L11 ANSWER 36 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 ACCESSION NUMBER: 1997:547160 SCISEARCH  
 THE GENUINE ARTICLE: XL484  
 TITLE: The LppC gene of *Streptococcus equisimilis* encodes a lipoprotein that is homologous to the e(P4) outer membrane protein from *Haemophilus influenzae*  
 AUTHOR: Gase K (Reprint); Liu G W; Bruckmann A; Steiner K; Ozegowski J; Malke H  
 CORPORATE SOURCE: UNIV JENA, INST MOL BIOL, D-07745 JENA, GERMANY  
 COUNTRY OF AUTHOR: GERMANY  
 SOURCE: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (JUN 1997) Vol. 186, No. 1, pp. 63-73.

ISSN: 0300-8584.  
PUBLISHER: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 39.  
ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We report the cloning, sequencing, and analysis of a novel chromosomal gene of *Streptococcus equisimilis* strain H46A that codes for a membrane lipoprotein, designated LppC. The lppC gene is located 3' adjacent to, and co-oriented with, the unrelated gapC gene that encodes the previously characterized glyceraldehyde-3-phosphate dehydrogenase. Sequencing of lppC revealed an 855-bp open reading frame that predicted a 32.4-kDa polypeptide possessing a potential lipoprotein signal sequence and modification site (VTGC). Signal sequence processing of LppC synthesized in the homologous host or expressed from plasmid pLPP2 in *Escherichia coli* was sensitive to globomycin, a selective inhibitor of lipoprotein-specific signal peptidase II. Subcellular localization of LppC using polyclonal antibodies raised to the hexahistidyl-tagged protein proved LppC to be tightly associated with the cytoplasmic membrane of *S. equisimilis* and with the outer membrane of *E. coli* JM109 (pLPP2). Southern, Northern and Western analyses indicated that Ipl, was conserved in *S. pyogenes*, and transcribed independently of gap as monocistronic 0.9-kb mRNA from a sigma(70)-like consensus promoter. Database searches found homology of LppC to the hel gene-encoded outer membrane protein e (P4) from *Haemophilus influenzae* to which it exhibits 58% sequence similarity. However, unlike the hel gene, lppC was unable to complement hemA mutants of *E. coli* for growth on hemin as sole porphyrin source in aerobic conditions. Furthermore, neither the wild type nor an lppC insertion mutant of *S. equisimilis* could grow on hemin in iron-limited medium. These results, together with findings indicating that *S. equisimilis* H46A had no absolute requirement for iron, led us to conclude that lppC, in contrast to hel, is not involved in hemin utilization and has yet to be assigned a function.

L11 ANSWER 37 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1997:155090 HCAPLUS  
DOCUMENT NUMBER: 126:154444  
TITLE: Streptokinases analogs resistant to cleavage and inactivation by plasmin  
INVENTOR(S): Reed, Guy L.  
PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA  
SOURCE: PCT Int. Appl., 64 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9641883	A1	19961227	WO 1996-US9640	19960607
W: CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5854049	A	19981229	US 1995-488940	19950609
PRIORITY APPLN. INFO.:			US 1995-488940	A 19950609

AB Streptokinase analogs with altered plasmin-binding features that are resistant to binding by plasmin and subsequent cleavage and inactivation are described for use as thrombolytics with a prolonged serum half-life. Specifically, analogs of the *Streptococcus equisimilis* streptokinase are described. Changes that increase plasminogen resistance include alterations of the plasmin-binding domain and and

blocking of the N-terminus. Fusion proteins with maltose-binding protein as the N-terminal moiety are prepared and their plasmin resistance and **streptokinase** activity are described. Similarly, analogs with substitutions of basic amino acids that identify internal plasmin and trypsin cleavage sites were prepared and characterized.

L11 ANSWER 38 OF 106 LIFESCI COPYRIGHT 2005 CSA on STN

ACCESSION NUMBER: 96:44932 LIFESCI

TITLE: Functional analysis of a relA/spoT gene homolog from *Streptococcus equisimilis*

AUTHOR: Mechold, U.; Cashel, M.; Steiner, K.; Gentry, D.; Malke, H.

CORPORATE SOURCE: Inst. Molecular Biol., Jena Univ., Winzerlaer Str. 10, D-07745 Jena, Germany

SOURCE: J. BACTERIOL., (1996) vol. 178, no. 5, pp. 1404-1411. ISSN: 0021-9193.

DOCUMENT TYPE: Journal

FILE SEGMENT: J; G

LANGUAGE: English

SUMMARY LANGUAGE: English

AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of *Streptococcus equisimilis* H46A. This gene, originally called rel, here termed rel sub( )*S. equisimilis*, is homologous to two related *Escherichia coli* genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of *E. coli* mutants led us to deduce that the highly **expressed** rel sub( )*S. equisimilis* gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel sub( )*S. equisimilis* protein, purified from an *E. coli* relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel sub( )*S. equisimilis* protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel sub( )*S. equisimilis* protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel sub( )*S. equisimilis* gene function in *E. coli* as being similar to that of the native spoT gene, disruptions of rel sub( )*S. equisimilis* in *S. equisimilis* abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L11 ANSWER 39 OF 106 MEDLINE on STN

DUPLICATE 12

ACCESSION NUMBER: 96200111 MEDLINE

DOCUMENT NUMBER: PubMed ID: 8631718

TITLE: Functional analysis of a relA/spoT gene homolog from *Streptococcus equisimilis*.

AUTHOR: Mechold U; Cashel M; Steiner K; Gentry D; Malke H

CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.

SOURCE: Journal of bacteriology, (1996 Mar) 178 (5) 1401-11. Journal code: 2985120R. ISSN: 0021-9193.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199607

ENTRY DATE: Entered STN: 19960715

Last Updated on STN: 19970203

Entered Medline: 19960703

AB We examined the functional attributes of a gene encountered by sequencing

the streptokinase gene region of Streptococcus equisimilis H46A. This gene, originally called rel, here termed relS. equisimilis, is homologous to two related Escherichia coli genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. coli mutants led us to deduce that the highly expressed rel S. equisimilis gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel S. equisimilis protein, purified from an E. coli relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel S. equisimilis protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel S. equisimilis protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel S. equisimilis gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel S. equisimilis in S. equisimilis abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L11 ANSWER 40 OF 106 MEDLINE on STN DUPLICATE 13  
 ACCESSION NUMBER: 96397500 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8804394  
 TITLE: Cloning of heterologous genes specifying detrimental proteins on pUC-derived plasmids in Escherichia coli.  
 AUTHOR: Muller J; van Dijl J M; Venema G; Bron S  
 CORPORATE SOURCE: Institut fur Molekularbiologie, Friedrich-Schiller-Universitat Jena, Germany.  
 SOURCE: Molecular & general genetics : MGG, (1996 Aug 27) 252 (1-2) 207-11.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199610  
 ENTRY DATE: Entered STN: 19961219  
 Last Updated on STN: 19961219  
 Entered Medline: 19961031

AB A system is described that enables the cloning of genes specifying detrimental proteins in Escherichia coli. The system is based on pUC plasmids and was developed for the expression of the Bacillus subtilis csaA gene, which is lethal when expressed at high levels. Suppressor strains that tolerate the presence of plasmids for high-level expression of csaA were isolated, which contained small cryptic deletion variants of the parental plasmid in high copy numbers. The cryptic plasmids consisted mainly of the pUC replication functions and lacked the csaA region and selectable markers. The co-resident, incompatible, cryptic plasmids enabled the maintenance of the csaA plasmids by reducing their copy number 20-fold, which resulted in a concomitant 3- to 7-fold reduction in the expression of plasmid-encoded genes. Strains carrying these cryptic endogenous plasmids proved to be useful for the construction of pUC-based recombinant plasmids carrying other genes, such as the skc gene of Streptococcus equisimilis, which cannot be cloned in high copy numbers in E. coli. Several strategies to reduce production levels of heterologous proteins specified by plasmids are compared.

L11 ANSWER 41 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN DUPLICATE 14

ACCESSION NUMBER: 96259705 EMBASE  
DOCUMENT NUMBER: 1996259705  
TITLE: Localization of the sequence-determined DNA bending center upstream of the **streptokinase** gene **skc**.  
AUTHOR: Gross S.; Gase K.; Malke H.  
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Winzerlaer Strasse 10, D-07745 Jena, Germany  
SOURCE: Archives of Microbiology, (1996) Vol. 166, No. 2, pp. 116-121.  
ISSN: 0302-8933 CODEN: AMICCW  
COUNTRY: Germany  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
037 Drug Literature Index  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 960924  
Last Updated on STN: 960924

AB DNA sequences upstream of the core promoter region of the **streptokinase** gene (**skc**) from *Streptococcus equisimilis* H46A increase **skc** transcription more than tenfold in vivo. This promoter upstream region contains a segment of intrinsically bent DNA, the precise location of which was determined experimentally by circular permutation analysis and theoretically by computer prediction. Electrophoretic analysis of circularly permuted upstream DNA fragments placed the bend center approximately at position -100 with respect to the major transcription initiation site of **skc**. This position was in excellent agreement with the center of maximum curvature predicted theoretically. Knowledge of the precise location of the bend center will be useful for future studies of the possible effect of DNA bending on **skc** transcription.

L11 ANSWER 42 OF 106 MEDLINE on STN DUPLICATE 15

ACCESSION NUMBER: 96305364 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8706717  
TITLE: Cloning, sequencing and functional overexpression of the *Streptococcus equisimilis* H46A gapC gene encoding a glyceraldehyde-3-phosphate dehydrogenase that also functions as a plasmin(ogen)-binding protein. Purification and biochemical characterization of the protein.  
AUTHOR: Gase K; Gase A; Schirmer H; Malke H  
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
SOURCE: European journal of biochemistry / FEBS, (1996 Jul 1) 239 (1) 42-51.  
Journal code: 0107600. ISSN: 0014-2956.  
PUB. COUNTRY: GERMANY: Germany; Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-X97788  
ENTRY MONTH: 199609  
ENTRY DATE: Entered STN: 19960919  
Last Updated on STN: 19990129  
Entered Medline: 19960910

AB We previously identified DNA sequences involved in the function of the complex promoter of the **streptokinase** gene from *Streptococcus equisimilis* H46A, a human serogroup C strain known to **express** this gene at a high level. As a prerequisite to understanding possible mechanisms that control the balance between the plasminogen activating and plasmin(ogen) binding capacities of H46A, we describe here its gapC gene encoding glyceraldehyde-3-phosphate

dehydrogenase (GraP-DH, EC 1.2.1.12), a glycolytic enzyme apparently transported to the cell surface where it functions as a plasmin(ogen).binding protein. The gapC gene was cloned and sequenced and found to code for a 336-amino-acid polypeptide (approximately 35.9 kDa) exhibiting 94.9% sequence identity to the Plr protein from Streptococcus pyogenes shown by others to be capable of plasmin binding [Lottenberg, R., Broder, C. C., Boyle, M. D., Kain, S. J., Schroeder, B. L. & Curtiss, R. III (1992) J. Bacteriol. 174, 5204-5210]. To study the properties of the GapC protein, its gene was inducibly overexpressed in Escherichia coli from QIAexpress expression plasmids to yield the authentic GapC or (His)6GapC carrying a hexahistidyl N-terminus to permit affinity purification. Both proteins were functionally active, exhibiting specific GraP-DH activities of about 80 kat/mol (approximately 130 U/mg) after purification. Their binding parameters [association (ka) and dissociation (kd) rate constants, and equilibrium dissociation constants (Kd = kd/ka)] for the interaction with human Gluplasminogen and plasmin were determined by real-time biospecific interaction analysis using the Pharmacia BIAcore instrument. For comparative purposes, the commercial GraP-DH from Bacillus stearothermophilus (BstGraP-DH), a nonpathogenic organism, was included in these experiments. The Kd values for binding of plasminogen to GapC, (His)6GapC and BstGraP-DH were 220 nM, 260 nM and 520 nM, respectively, as compared to 25 nM, 17 nM and 98 nM, respectively, for the binding to plasmin. These data show that both the zymogen and active enzyme possess low-affinity binding sites for the gapC gene product and that the hexahistidyl terminus does not affect its function. Prior limited treatment with plasmin enhanced the subsequent plasminogen binding capacity of all three GraP-DHs, presumably by the exposure of new C-terminal lysine residues for binding to the zymogen.

L11 ANSWER 43 OF 106 MEDLINE on STN DUPLICATE 16  
 ACCESSION NUMBER: 96396845 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8803948  
 TITLE: Structural dissection and functional analysis of the complex promoter of the streptokinase gene from Streptococcus equisimilis H46A.  
 AUTHOR: Grafe S; Ellinger T; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Medical microbiology and immunology, (1996 May) 185 (1) 11-7.  
 Journal code: 0314524. ISSN: 0300-8584.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199701  
 ENTRY DATE: Entered STN: 19970219  
 Last Updated on STN: 19970219  
 Entered Medline: 19970131  
 AB The overlapping tandem promoters of the streptokinase gene, P1 and P2, identified previously by S1 nuclease transcript mapping were functionally dissected by mutagenesis of their -10 regions and fused transcriptionally with or without the 202-bp upstream region (USR) to the luciferase reporter gene (luc) from Photinus pyralis to analyze the contribution of the different sequence elements to promoter activity in Escherichia coli and the homologous Streptococcus equisimilis strain H46A. In E. coli, virtually the entire promoter activity derived from the upstream promoter P1. In S. equisimilis, luc expression increased in the following order of the involved sequence elements: P2 approximately equal to P2 + USR < P1 < P1 + P2 < P1 + USR < P1 + P2 + USR. This shows that (1) in the homologous system, P1 and P2 alone are extremely weak, (2) in the USR-less arrangement, only the combined core promoters have substantial activity, and (3) the USR stimulates only P1 and the combination of P1 + P2. Thus, the tandem

promoters presumably function by mutual contributory action and their full activity strongly depends on the AT-rich and statically bent upstream region. The distinctive feature determining the strength of P1 in both hosts appears to be its extended -10 region which matches the consensus TRTGN established for strong *S. pneumoniae* and *Bacillus subtilis* promoters.

L11 ANSWER 44 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:433656 HCAPLUS  
 DOCUMENT NUMBER: 133:27355  
 TITLE: Cloning and expression of Streptococcus H46 streptokinase gene  
 INVENTOR(S): Cho, Jung-Myong; Park, Yong-U.  
 PATENT ASSIGNEE(S): LG Chemical Co., Ltd., S. Korea  
 SOURCE: Repub. Korea, No pp. given  
 CODEN: KRXXFC  
 DOCUMENT TYPE: Patent  
 LANGUAGE: Korean  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 9512901	B1	19951023	KR 1992-17406	19920924
PRIORITY APPLN. INFO.:			KR 1992-17406	19920924

AB The cloning of streptokinase gene of Streptococcus H46 consists of PCR with primers and cloning the gene into the PstI-NdeI site of plasmid ptrp322H-HGH (KFCC 10067) to get ptrpH-SK (ATCC 68884). The DNA sequence of Streptococcus H46 streptokinase has 92.2-98.8% homol. to SKC, SKG, and SKA. Streptococcus H46 is also designated *S. equisimilis* ATCC 35556.

L11 ANSWER 45 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1996:90928 BIOSIS  
 DOCUMENT NUMBER: PREV199698663063  
 TITLE: Genetic modification of the streptokinase gene to study the interaction of streptokinase with plasminogen.  
 AUTHOR(S): Lizano, S.; Johnston, K. H. [Reprint author]  
 CORPORATE SOURCE: Dep. Microbiol. Immunol. Parasitol., La. State Univ. Med. Cent., 1901 Perdido St., New Orleans, LA 70112, USA  
 SOURCE: Ferretti, J. J. [Editor]; Klaenhammer, T. R. [Editor]; Brown, F. [Editor]; Gilmore, M. S. [Editor]. Dev. Biol. Stand., (1995) pp. 195-198. Developments in Biological Standardization; Genetics of streptococci, enterococci and lactococci.  
 Publisher: S. Karger AG, P.O. Box, Allschwilerstrasse 10, CH-4009 Basel, Switzerland; S. Karger AG, New York, New York, USA. Series: Developments in Biological Standardization.  
 Meeting Info.: 4th International American Society for Microbiology Conference on Streptococcal Genetics. Santa Fe, New Mexico, USA. May 15-18, 1994.  
 CODEN: DVBSA3. ISSN: 0301-5149. ISBN: 3-8055-6207-1.  
 DOCUMENT TYPE: Book  
 Conference; (Meeting)  
 Book; (Book Chapter)  
 Conference; (Meeting Paper)  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 4 Mar 1996  
 Last Updated on STN: 4 Mar 1996

L11 ANSWER 46 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on



STN

ACCESSION NUMBER: 1996:90927 BIOSIS  
DOCUMENT NUMBER: PREV199698663062  
TITLE: The **streptokinase** gene: Allelic variation,  
genomic environment and **expression** control.  
AUTHOR(S): Malke, H. [Reprint author]; Steiner, K.; Gase, K.; Mechold,  
U.; Ellinger, T.  
CORPORATE SOURCE: Inst. Mol. Biol., Jena Univ., Winzerlaer Str. 10, D-07745  
Jena, Germany  
SOURCE: Ferretti, J. J. [Editor]; Klaenhammer, T. R. [Editor];  
Brown, F. [Editor]; Gilmore, M. S. [Editor]. Dev. Biol.  
Stand., (1995) pp. 183-193. Developments in Biological  
Standardization; Genetics of streptococci, enterococci and  
lactococci.  
Publisher: S. Karger AG, P.O. Box, Allschwilerstrasse 10,  
CH-4009 Basel, Switzerland; S. Karger AG, New York, New  
York, USA. Series: Developments in Biological  
Standardization.  
Meeting Info.: 4th International American Society for  
Microbiology Conference on Streptococcal Genetics. Santa  
Fe, New Mexico, USA. May 15-18, 1994.  
CODEN: DVBSA3. ISSN: 0301-5149. ISBN: 3-8055-6207-1.  
DOCUMENT TYPE: Book  
Conference; (Meeting)  
Book; (Book Chapter)  
Conference; (Meeting Paper)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 4 Mar 1996  
Last Updated on STN: 4 Mar 1996

L11 ANSWER 47 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1995:547506 BIOSIS  
DOCUMENT NUMBER: PREV199698561806  
TITLE: High-level **expression** and secretion of  
**streptokinase** in Escherichia coli.  
AUTHOR(S): Ko, Jae Hyeong; Park, Do Deun; Kim, Il Chul; Lee, Si  
Hyoung; Byun, Si Myung [Reprint author]  
CORPORATE SOURCE: Dep. BioSci., Korea Advanced Inst. Sci. Technol., 373-1,  
Kusung-dong, Yusung-ku, Taejeon 305-701, South Korea  
SOURCE: Biotechnology Letters, (1995) Vol. 17, No. 10, pp.  
1019-1024.  
CODEN: BILED3. ISSN: 0141-5492.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 31 Dec 1995  
Last Updated on STN: 31 Dec 1995

AB The high-level **expression** plasmid for **streptokinase**,  
pSK100, has been constructed. It contains a tac promoter, an ompA signal  
sequence, a **streptokinase** structural gene(**skc**) and a  
rrnBT1T2 transcription terminator. E. coli JM 109 carrying pSK100  
produced about 5,000IU of **streptokinase** per 1 ml of  
LB-ampicillin media. About 95% of the **expressed**  
**streptokinase** was secreted into the periplasmic and extracellular  
fractions. The **recombinant streptokinase** in high  
yield and purity may be a potential alternative source for the therapeutic  
agent.

L11 ANSWER 48 OF 106 MEDLINE on STN DUPLICATE 17

ACCESSION NUMBER: 95342169 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 7616967  
TITLE: Complex transcriptional control of the  
**streptokinase** gene of Streptococcus  
**equisimilis** H46A.

AUTHOR: Gase K; Ellinger T; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Molecular & general genetics : MGG, (1995 Jun 25) 247 (6) 749-58.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199508  
 ENTRY DATE: Entered STN: 19950905  
 Last Updated on STN: 19950905  
 Entered Medline: 19950822

AB On the *Streptococcus equisimilis* H46A chromosome, the divergent coding sequences of the genes for the plasminogen activator **streptokinase** (**skc**) and a leucine-rich protein (**lrp**), the function of which is unknown, are separated by a 328 bp intrinsically bent DNA region rich in AT tracts. To begin to understand the expression control of these two genes, we mapped their transcriptional initiation sites by S1 nuclease analysis and studied the influence of the bent intergenic region on promoter strength, using promoter-reporter gene fusions of **skc'** and **lrp'** to '**lacZ** from *Escherichia coli*. The major transcriptional start sites, in both *S. equisimilis* and *E. coli*, mapped 22 bases upstream of the ATG start site of **lrp** (G), and 24 and 32 bases upstream of the translational initiation codon of **skc** (A and G, respectively), indicating the existence of two overlapping canonical **skc** promoters arranged in tandem on opposite faces of the helix. The reporter gene fusions were cloned in *E. coli* on a vector containing a 1.1 kb fragment of the *S. equisimilis* **dexB** gene, thus allowing promoter strength to be measured in multiple plasmid-form copies in the heterologous host and in single-copy genomic form following integration into the **skc** region of the homologous host. In *S. equisimilis*, **skc** '**lacZ** was expressed about 200-fold more strongly than the corresponding **lrp'**-**lacZ** fusion. In contrast, in *E. coli*, the corresponding levels of expression differed by only about 11-fold. Deletion of the 202 bp bent region upstream of the **skc** and **lrp** core promoters caused a 13-fold decrease in **skc** promoter activity in *S. equisimilis* but did not alter **lrp** promoter strength in this host. In contrast, when studied in *E. coli*, this deletion did not alter the strength of the **skc**-double promoter and even increased by 2.4- to 3-fold the activity of the **lrp** promoter. This comparative promoter analysis shows that **skc** has a complex promoter structure, the activity of which in the homologous genomic environment specifically depends on sequences upstream of the two core promoters. Thus, the **skc** promoter structure resembles that of an array of promoters involved in a transcriptional switch; however, the nature of the potential switch factor(s) remains unknown.

L11 ANSWER 49 OF 106 MEDLINE on STN DUPLICATE 18  
 ACCESSION NUMBER: 95157528 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 7531815  
 TITLE: Transcription termination of the **streptokinase** gene of *Streptococcus equisimilis* H46A: bidirectionality and efficiency in homologous and heterologous hosts.  
 AUTHOR: Steiner K; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Molecular & general genetics : MGG, (1995 Feb 6) 246 (3) 374-80.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English

FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199503  
ENTRY DATE: Entered STN: 19950322  
Last Updated on STN: 19960129  
Entered Medline: 19950316

AB In *Streptococcus equisimilis* H46A, a hypersymmetrical transcription terminator with bidirectional activity was localized between the translational termination codons of the *streptokinase* gene, *skc*, and the *rel-orf1* genes. These two transcription units are oriented towards each other, and under normal conditions the *skc* mRNA level exceeds that of the *rel-orf1* genes by a factor of at least 1000. Reporter vectors based on the promoterless *cat* gene were constructed by transcriptional fusion of *skc* to *cat*, such that the region between the two genes contained the terminator in *skc* orientation or in *rel-orf1* orientation. Additionally, *skc* and *cat* were fused directly, with deletion of the terminator. The reporter vectors were designed to be capable of being studied either as multicopy plasmids in *Escherichia coli* or in single copy following integration, via *skc*, into the *S. equisimilis* chromosome. Chloramphenicol acetyl transferase (CAT) activity assays in conjunction with determination of chloramphenicol resistance levels and Northern hybridization analysis showed that the terminator is active in either host and orientation. However, termination efficiency was host dependent, with high terminator strength being observed in the homologous streptococcal background and appreciable readthrough occurring in *E. coli*. The extent of transcriptional readthrough was dependent upon terminator orientation, with termination being more efficient in *rel-orf1* polarity. The results suggest that, in *S. equisimilis*, transcription of both *skc* and *rel-orf1* is efficiently terminated by a common signal, and that these genes are largely protected from convergent transcription, which otherwise would seem to be particularly detrimental to the weakly expressed *rel-orf1* genes.

L11 ANSWER 50 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation  
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ACCESSION NUMBER: 1995:622543 SCISEARCH  
THE GENUINE ARTICLE: RU780  
TITLE: STREPTOKINASE-MEDIATED PLASMINOGEN ACTIVATION  
USING A RECOMBINANT DUAL FUSION PROTEIN  
CONSTRUCT - A NOVEL-APPROACH TO STUDY BACTERIAL HOST  
PROTEIN INTERACTIONS  
AUTHOR: LIZANO S (Reprint); JOHNSTON K H  
CORPORATE SOURCE: LOUISIANA STATE UNIV, MED CTR, DEPT MICROBIOL IMMUNOL &  
PARASITOL, NEW ORLEANS, LA 70112  
COUNTRY OF AUTHOR: USA  
SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (SEP 1995) Vol. 23,  
No. 3, pp. 261-280.  
ISSN: 0167-7012.  
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
NETHERLANDS.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 59  
ENTRY DATE: Entered STN: 1995  
Last Updated on STN: 1995

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB *Streptokinase* (SK), a plasminogen (Pg) activator secreted by groups A, C, and G streptococci, is extensively used as a pharmacological agent in thrombolytic therapy and possibly plays a role in streptococcal invasiveness and disease. SK activates Pg to plasmin (Ps) by forming an activator complex with Pg. However, the molecular basis whereby SK binds and activates Pg remains unclear, in part due to the rapid fragmentation of the SK-Pg complex. This study describes a solid phase approach to

study this interaction in which a recombinant SK molecule was constructed with glutathione-S-transferase appended to the NH2 terminus and (Gly)(3)(His)(8) appended to the COOH terminus. This dual fusion protein molecule, immobilized on either Sepharose-S-hexylglutathione or Ni2+ dinitriloacetic acid-Sepharose was then used to study the interaction of SK with Pg. These SK-Pg complexes exhibited amidolytic and proteolytic activity similar to native SK, but the pattern of fragmentation of the SK molecule was dependent upon whether the SK molecule was immobilized either at its NH2- or COOH terminus. This solid phase approach may contribute to a greater understanding of the role of SK in Pg activation by enabling the 'capture' of intact activator complexes under physiological conditions and, in addition, may serve as a useful model to analyze other bacterial-host protein interactions important in the pathogenesis of disease.

L11 ANSWER 51 OF 106 MEDLINE on STN DUPLICATE 19  
 ACCESSION NUMBER: 96157065 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8586173  
 TITLE: The **streptokinase** gene: allelic variation, genomic environment and **expression** control.  
 AUTHOR: Malke H; Steiner K; Gase K; Mechold U; Ellinger T  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Developments in biological standardization, (1995) 85 183-93. Ref: 36  
 Journal code: 0427140. ISSN: 0301-5149.  
 PUB. COUNTRY: Switzerland  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 General Review; (REVIEW)  
 (REVIEW, TUTORIAL)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199603  
 ENTRY DATE: Entered STN: 19960404  
 Last Updated on STN: 19960404  
 Entered Medline: 19960328  
 AB The genes for **streptokinase**, the most important prokaryotic plasminogen activator, exhibit allelic variation predominantly due to the polymorphism of an internal 220-base pair fragment that divides the phylogenetic tree of their products into two primary branches. Current molecular genetic research seeks functional correlates of the allelic variation, aims at analyzing the genomic environment of the **streptokinase** gene, **skc**, and focuses on understanding its **expression**. Of the six genes cloned and sequenced in the **skc** region of *Streptococcus equisimilis* H46A, **skc** is **expressed** most abundantly in a fashion that involves two overlapping core promoters and upstream sequences rich of AT tracts. Transcription of **skc** is terminated at a hypersymmetrical site that functions bidirectionally and prevents convergent transcription of the oppositely oriented **skc** and **rel-orf1** genes whose mRNA abundance differs by a factor of at least three orders of magnitude.

L11 ANSWER 52 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 ACCESSION NUMBER: 1995:290311 BIOSIS  
 DOCUMENT NUMBER: PREV199598304611  
 TITLE: Cloning, **Expression** and Sequence Analysis of **Streptokinases** from *Streptococcus equisimilis* which Preferentially Activate Equine and Porcine Plasminogens.  
 AUTHOR(S): Caballero, A. [Reprint author]; Johnston, K. H. [Reprint author]; Lottenberg, R.  
 CORPORATE SOURCE: LSU Med. Ctr., New Orleans, LA, USA  
 SOURCE: Abstracts of the General Meeting of the American Society

for Microbiology, (1995) Vol. 95, No. 0, pp. 172.  
Meeting Info.: 95th General Meeting of the American Society  
for Microbiology. Washington, D.C., USA. May 21-25, 1995.  
ISSN: 1060-2011.

DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 5 Jul 1995  
Last Updated on STN: 5 Jul 1995

L11 ANSWER 53 OF 106 MEDLINE on STN DUPLICATE 20

ACCESSION NUMBER: 96154934 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8577315  
TITLE: Conservation of the organization of the  
**streptokinase** gene region among pathogenic  
streptococci.  
AUTHOR: Frank C; Steiner K; Malke H  
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
SOURCE: Medical microbiology and immunology, (1995 Oct) 184 (3)  
139-46.  
Journal code: 0314524. ISSN: 0300-8584.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-X72832  
ENTRY MONTH: 199603  
ENTRY DATE: Entered STN: 19960321  
Last Updated on STN: 19960321  
Entered Medline: 19960313

AB Using ten gene-specific probes from the cloned and sequenced  
**streptokinase** gene (*skc*) region (8,931 bp) of  
*Streptococcus equisimilis* H46A, a human serogroup C strain, the  
conservation of these genes and their linkage relationships were studied  
by Southern hybridization in pathogenic streptococci differing  
taxonomically, serologically, in regard to their host range, and in the  
class of plasminogen activator produced. The results indicate that in *S.*  
*pyogenes* (strains A374, NZ131 and SF130/13) and a human group G strain  
(G19,908) both gene content and gene order as determined for H46A  
(*dexB-abc-lrp-skc-orf1-rel*) are preserved. The same is true of  
an equine *S. equisimilis* isolate (87-542-W), the  
**streptokinase** gene of which has been shown to hybridize detectably  
with *skc*, a result at variance with that obtained previously by  
others. In contrast, the chromosomal DNA of three *S. uberis* strains  
(0140J, C198, C216) of bovine origin, two of which produced a plasminogen  
activator different from **streptokinase**, hybridized only with  
*dexB-*, *abc-* and *rel*-specific probes, and the homologues of these genes  
appeared to lie close to each other. The maintenance of the organization  
of the **streptokinase** gene region in strains differing in overall  
chromosomal character suggests that this gene arrangement is of selective  
advantage.

L11 ANSWER 54 OF 106 MEDLINE on STN DUPLICATE 21

ACCESSION NUMBER: 96001243 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 7557478  
TITLE: Secretion of **streptokinase** fusion proteins from  
*Escherichia coli* cells through the hemolysin transporter.  
AUTHOR: Kern I; Ceglowski P  
CORPORATE SOURCE: Institute of Biochemistry and Biophysics, Polish Academy of  
Sciences, Warszawa.  
SOURCE: Gene, (1995 Sep 22) 163 (1) 53-7.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199511  
ENTRY DATE: Entered STN: 19951227  
Last Updated on STN: 20021008  
Entered Medline: 19951108

AB The hemolysin (HlyA) secretion system was used to achieve the sec-independent secretion of **streptokinase (Skc)** originating from *Streptococcus equisimilis* into the medium by *Escherichia coli* cells. The in-frame fusions of the **skc** gene, either possessing or lacking a region encoding the signal peptide (SP) with the 3'-end of the **hlyA** gene of various lengths were analysed. All hybrids retained **Skc** activity. Hybrid proteins devoided of the N-terminal SP, regardless of length of the **hlyA** secretion signal (62 vs. 194 amino acids), were secreted into the medium by the *E. coli* HlyA transporter at similar levels. Considerable amounts of hybrid proteins were still, however, associated with *E. coli* cells, mainly in the degraded form.

L11 ANSWER 55 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1996:43901 SCISEARCH  
THE GENUINE ARTICLE: TN446  
TITLE: Cloning and sequencing of the **streptokinase** gene from *Streptococcus pyogenes* (CIP 56.57)  
AUTHOR: Ball M M (Reprint); Puig J; Iborra F  
CORPORATE SOURCE: UNIV PARIS 11, F-91405 ORSAY, FRANCE  
COUNTRY OF AUTHOR: FRANCE  
SOURCE: DNA SEQUENCE, (1995) Vol. 6, No. 1, pp. 33-36. ISSN: 1042-5179.  
PUBLISHER: HARWOOD ACAD PUBL GMBH, C/O STBS LTD, PO BOX 90, READING, BERKS, ENGLAND RG1 8JL.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 10  
ENTRY DATE: Entered STN: 1996  
Last Updated on STN: 1996

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The **streptokinase** gene of the *Streptococcus pyogenes* strain CIP 56.57 was cloned and sequenced. This sequence coding for a 441 amino acid protein is well conserved among streptococcus species: there are two very conserved domains separated by a more variable region.

L11 ANSWER 56 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
DUPLICATE 22

ACCESSION NUMBER: 1994-07329 BIOTECHDS  
TITLE: DNA encoding a plasminogen binding protein; **recombinant streptokinase** fragment production using new vector plasmid pMAL and a monoclonal antibody for use in myocardial infarction therapy  
PATENT ASSIGNEE: Gen.Hosp.Boston; Univ.Harvard  
PATENT INFO: WO 9407992 14 Apr 1994  
APPLICATION INFO: WO 1993-US9502 5 Oct 1993  
PRIORITY INFO: US 1993-128299 29 Sep 1993; US 1992-956692 5 Oct 1992  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 1994-135561 [16]

AB DNA (I) encoding a **streptokinase** fragment (II) of residues 14-414 of a disclosed protein sequence is claimed. (II) does not contain residues 244-352, but may contain residues 1-352, 120-352, 244-414 or 244-352 of the protein sequence. Also claimed are: (1) an **expression** vector containing (I); (2) a host cell transformed

with the vector of (1); (3) (II) encoded by (I); (4) detecting plasminogen in a biological sample by contacting the sample with (II) and detecting any (II)-plasminogen complex formed; (5) a method for assaying (II) for antigenicity involving contacting (II) with a monoclonal antibody specific for a distinct epitope of **streptokinase** and determining whether the fragments bind to the MAb and, optionally, also whether the fragments can activate plasminogen in the presence of the MAb; (6) a method for myocardial infarction therapy involving administering (II) to a patient; and (7) a monoclonal antibody specific for a distinct epitope of **streptokinase**. In an example, (II) genes from *Streptococcus equisimilis* were fused with maltose binding protein genes and **expressed** in *Escherichia coli* using plasmid pMAL. (62pp)

L11 ANSWER 57 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
DUPLICATE 23

ACCESSION NUMBER: 1994-04368 BIOTECHDS

TITLE: Engineering and production of **streptokinase** in a  
Bacillus subtilis **expression**-secretion system;  
Streptococcus **equisimilis** gene cloning  
, protein secretion and protein engineering for improved  
C-terminus stability in a protease-deficient strain

AUTHOR: Wong S L; Ye R; Nathoo S

CORPORATE SOURCE: Univ.Calgary

LOCATION: Department of Biological Sciences, The University of Calgary,  
2500 University Drive, N.W., Calgary, Alberta T2N 1N4,  
Canada.

SOURCE: Appl.Environ.Microbiol.; (1994) 60, 2, 517-23  
CODEN: AEMIDF

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Production of *Streptococcus equisimilis* H46A secreted  
**recombinant streptokinase** from various *Bacillus*  
*subtilis* strains was studied. The **skc** gene was cloned  
and **expressed** using plasmid pSK-1 (with the intact **skc**  
gene and its promoter region) or plasmid pSK-3 (with the mature portion  
of **streptokinase** fused to the *B. subtilis* levansucrase **sacB**  
gene promoter and signal peptide sequence) as vector. The use of the  
6-extracellular-protease-deficient strain *B. subtilis* WB600 as host  
greatly improved the production yield. *B. subtilis* carrying either the  
wild-type or modified **skc** gene produced **streptokinase**  
at a comparable level. Even with WB600 as host, a C-terminally-processed  
**streptokinase** was also produced. Through region-specific  
combinatorial mutagenesis around the C-terminal processing sites,  
**streptokinase** derivatives resistant to C-terminal degradation  
were engineered. Plasmid pSKC-27 encoded a derivative which showed a  
2.5-fold increase in specific activity, which should make it a more  
favorable thrombolytic agent. (35 ref)

L11 ANSWER 58 OF 106 MEDLINE on STN DUPLICATE 24

ACCESSION NUMBER: 94178706 MEDLINE

DOCUMENT NUMBER: PubMed ID: 8132150

TITLE: Inactivation of the **streptokinase** gene prevents  
*Streptococcus equisimilis* H46A from acquiring  
cell-associated plasmin activity in the presence of  
plasminogen.

AUTHOR: Malke H; Mechold U; Gase K; Gerlach D

CORPORATE SOURCE: Institute for Molecular Biology, Jena University, FRG.

SOURCE: FEMS microbiology letters, (1994 Feb 1) 116 (1) 107-12.  
Journal code: 7705721. ISSN: 0378-1097.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199404  
ENTRY DATE: Entered STN: 19940428  
Last Updated on STN: 19940428  
Entered Medline: 19940418

AB The **streptokinase** gene of *Streptococcus equisimilis* H46 was inactivated by plasmid insertion mutagenesis to study the relationship between elaboration of **streptokinase** and acquisition of cell-associated plasmin activity after incubation of wild-type and mutant cells in media containing plasminogen or plasmin. The results showed that H46A binds both the zymogen and active enzyme, generates surface-associated plasmin activity in the presence of plasminogen when producing **streptokinase**, and **expresses** its plasmin(ogen) receptor(s) independently of a functional **streptokinase** gene. At least part of the plasmin(ogen) binding capacity may be due to the glyceraldehyde-3-phosphate dehydrogenase type of receptor molecule, as judged by the detection of the corresponding gene.

L11 ANSWER 59 OF 106 MEDLINE on STN DUPLICATE 25  
ACCESSION NUMBER: 94049672 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8232196  
TITLE: Genetic organization of the **streptokinase** region of the *Streptococcus equisimilis* H46A chromosome.  
AUTHOR: Mechold U; Steiner K; Vettermann S; Malke H  
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
SOURCE: Molecular & general genetics : MGG, (1993 Oct) 241 (1-2) 129-40.  
Journal code: 0125036. ISSN: 0026-8925.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-X72832  
ENTRY MONTH: 199312  
ENTRY DATE: Entered STN: 19940117  
Last Updated on STN: 19940117  
Entered Medline: 19931215

AB The complete nucleotide sequences of four genes and one open reading frame (ORF1) adjacent to the **streptokinase** gene, **skc**, from *Streptococcus equisimilis* H46A were determined. These genes are encoded on the opposite DNA strand to **skc** and are arranged as follows: dexB-abc-lrp-**skc**-ORF1-rel. The dexB gene, coding for an alpha-glucosidase (M(r) 61,733), and abc, encoding an ABC transporter (M(r) 42,080), are similar to the dexB and msmK genes, respectively, from the multiple sugar metabolism operon of *S. mutans*. The lrp gene specifies a leucine-rich protein (M(r) 32,302) that has a leucine-zipper motif at its C-terminus. The function of the Lrp protein is not known but appeared to be detrimental when overexpressed in *Escherichia coli*. Although lrp appears not to be an essential gene, as judged by plasmid insertion mutagenesis, it is conserved in all streptococcal strains carrying a **streptokinase** gene. The rel gene showed significant homology to the *E. coli* relA and spoT genes involved in the stringent response to amino acid deprivation. Multiple alignment of the amino acid sequences of Rel (M(r) 83,913), RelA and SpoT revealed 59.4% homology of the primary structures. Northern hybridization analyses of the genes in the **skc** region showed **skc** to be transcribed most abundantly. In addition to transcripts for **skc**, monocistronic mRNAs were detected for all three genes divergently transcribed from **skc**. Although there was also some read-through transcription from lrp into abc, and from abc into dexB, the transcription pattern suggests a high degree of transcriptional and functional independence not only of **skc** but also abc and dexB. Prominent structural features in intergenic regions included a static DNA bending locus located upstream and a putative bidirectional transcription terminator downstream of **skc**.



L11 ANSWER 60 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:441935 HCAPLUS

DOCUMENT NUMBER: 117:41935

TITLE: Cloning and expression of  
streptokinase gene of C-type Streptococcus  
equisimilis

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB),  
Cuba

SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04030794	A2	19920203	JP 1990-201600	19900731
JP 3127298	B2	20010122		
EP 489201	A1	19920610	EP 1990-201930	19900717
EP 489201	B1	19951115		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 130369	E	19951215	AT 1990-201930	19900717
ES 2081909	T3	19960316	ES 1990-201930	19900717
US 5296366	A	19940322	US 1991-703778	19910522
AU 644657	B2	19931216	AU 1991-78101	19910531
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A 19900523
			SU 1991-5001280	A 19910717

AB The streptokinase (I) gene SKC-2  
,with/without signal sequence, is cloned from C-type S.  
equisimilis ATCC-9542 by the polymerase chain reaction method and  
expressed in Escherichia coli and yeast for com. manufacture of I.  
Genomic DNA of the C-type S. equisimilis was isolated by the  
standard method and amplified with primers derived from the nucleotide  
sequence of SKC to get I gene with/without signal sequence.  
Expression of the I gene in E. coli and Pichia pastoris MP-36  
mutant were shown. The production of I with these microorganisms were  
≥350 mg/L and ≥1.2 g/L, resp.

L11 ANSWER 61 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1993:229181 HCAPLUS

DOCUMENT NUMBER: 118:229181

TITLE: Cloning and expression of a gene  
for streptokinase from a hemolytic  
Streptococcus

INVENTOR(S): Garcia, Mario P. E.; Chaplen, Roger R.; Hidalgo, Aimee  
P.; Doce, Ricardo S.; Marrero, Luciano F. H.; Collazo,  
Pedro R.; Ramirez, Anaisel C.; Munoz, Emilio A. M.;  
Martinez, Walfrido B.; et al.

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB),  
Cuba

SOURCE: Can. Pat. Appl., 26 pp.

CODEN: CPXXEB

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2043953	AA	19921206	CA 1991-2043953	19910605

CA 2043953	C	20010612		
HU 62655	A2	19930528	HU 1991-1770	19910527
HU 216073	B	19990428		
SK 279873	B6	19990507	SK 1991-2256	19910719
PRIORITY APPLN. INFO.:			CA 1991-2043953	19910605

AB The gene for **streptokinase** of a *Streptococcus equisimilis* type C is cloned and expressed in *Escherichia coli* or in yeasts. Expression in yeasts uses the promoter of the AOX1 gene of *Pichia pastoris* to regulate expression. Secretion of the protein was achieved using the cognate signal peptide or one from sucrose invertase. The gene was cloned by PCR amplification using different pairs of primers to clone the gene with or without the signal sequence. Integrating expression vectors for expression of the gene in *Pichia pastoris* with or without secretion of the product were constructed. When the secretory construct was used, **streptokinase** yields of 1-1.2 g/L were obtained. The protein had the expected biol. activities, and the purified enzyme had a specific activity of 50,000-100,000 units/mg.

L11 ANSWER 62 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1992:340953 BIOSIS  
DOCUMENT NUMBER: PREV199243030503; BR43:30503  
TITLE: **STREPTOKINASE MUTATIONS AFFECTING SKC EXPRESSION IN HOMOLOGOUS AND HETEROLOGOUS HOSTS.**  
AUTHOR(S): MECHOLD U [Reprint author]; MULLER J; MALKE H  
CORPORATE SOURCE: CENTRAL INST MICROBIOL EXP THERAPY, JENA D-6900, GER  
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1992) pp. 336-338. OREFICI, G. (ED.). ZENTRALBLATT FUER BAKTERIOLOGIE SUPPLEMENT, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS; (INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS); XI LANCEFIELD INTERNATIONAL SYMPOSIUM ON STREPTOCOCCI AND STREPTOCOCCAL DISEASES, SIENA, ITALY, SEPTEMBER 10-14, 1990. XIX+569P. GUSTAV FISCHER VERLAG: STUTTGART, GERMANY; NEW YORK, NEW YORK, USA. ILLUS.  
Publisher: Series: Zentralblatt fuer Bakteriologie Supplement.  
ISSN: 0941-018X. ISBN: 3-437-11362-3, 1-56081-333-4.  
DOCUMENT TYPE: Book  
Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 16 Jul 1992  
Last Updated on STN: 16 Jul 1992

L11 ANSWER 63 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
DUPLICATE 26

ACCESSION NUMBER: 1992-13545 BIOTECHDS  
TITLE: High level **expression** of **streptokinase** in *Escherichia coli*;  
gene cloning, expression and purification of thrombolytic protein  
AUTHOR: Estrada M P; Hernandez L; Perez A; Rodriguez P; \*de la Fuente J; Herrera L  
LOCATION: Mammalian Cell Genetics Division, Centro de Ingenieria Genetica y Biotecnologia, P.O. Box 6162, Havana 6, Cuba.  
SOURCE: Bio/Technology; (1992) 10, 10, 1138-42  
CODEN: BTCHDA  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The **streptokinase** (SK) gene was isolated by the polymerase chain reaction from *Streptomyces equisimilis* ATCC 9542. The

5-amplification primer introduced an ATG codon for translation initiation in *Escherichia coli*. The amplified fragment, which lacked the signal peptide sequence, was digested with BamHI, inserted into vector plasmid pTrp (to obtain plasmid pEKG-3 containing the SK gene under the control of a trp promoter), and used to transform *E. coli* HB101 cells. The DNA sequence of the SK gene region contained 5 differences at the amino acid level with respect to the reported SK protein. Plasmid pEKG-3 was introduced into *E. coli* K-12 strain W3110 for **expression**. The trp promoter was induced, and maximal SK **expression** was obtained after 14 hr, at which time the plasmid copy number reached 420 copies/cell. The **recombinant** SK was found in the cell cytosol and constituted 25% of total cell protein. It was purified by affinity chromatography using acylated human plasminogen coupled to Sepharose-4B, and ionexchange chromatography on DEAE-Sephacel. The **recombinant** product and natural SK had equivalent biological activities. (38 ref)

L11 ANSWER 64 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:646505 HCAPLUS

DOCUMENT NUMBER: 117:246505

TITLE: **Streptokinase** mutation affecting **skc expression** in homologous and heterologous hosts

AUTHOR(S): Mechold, U.; Muller, J.; Malke, H.

CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Jena, D-6900, Germany

SOURCE: Zentralblatt fuer Bakteriologie, Supplement (1992), 22(New Perspect. Streptococci Streptococcal Infect.), 336-8

CODEN: ZBASE2; ISSN: 0941-018X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Mutations affecting the level of **streptokinase** gene **skc expression** and/or secretion in homologous and heterologous hosts are phys. characterized. The principal classes of mutations produced included **skc** deletions, IS element insertions, and **skc** duplications. The deletion events, represented by mutations  $\Delta(\text{skc})$ -247 and  $\Delta(\text{skc})$ -305 present in plasmids pMM247 and pMM305, resp., removed a tetrapeptide (F10-L13 or L12-A15) from the hydrophobic core of the Skc signal sequence. These mutations, reduced the size, hydrophobicity and predicted alpha-helicity of the central region of the signal sequence. The corresponding plasmids, upon transformation into *E. coli* and *P. mirabilis* L-forms, substantially increased the level of **Skc expression** in either host. In *E. coli*, they also facilitated the export of mature **Skc** into the culture medium. In the gram-pos. hosts, **skc expression** was less dramatically affected; however, the proportion of **Skc** activity found in the culture medium was significantly decreased when compared to the extracellular activity resulting from wild type **skc**. IS1 insertion did not alter the primary structure of the promoter but displaced in upward direction, by 768 bp, a static DNA bending locus having its center some 140 bp upstream of the -35 region in wild type DNA. When studied with plasmid pMM697, this insertion event resulted in severely decreased **Skc expression** in all hosts but, expectedly, did not affect **Skc** secretability. Gene **skc** duplication in the chromosome of the homologous producer strain, *S. equisimilis* H46A, was achieved by a single crossover event between the chromosomes and an integrateable **Skc** plasmid, pSM752, in the region of shared homol. As judged by Southern hybridization, cells transiently supporting the replication of pSM752 gave rise to a stable erythromycin-resistant clone designated H46SM which was plasmid-free and produced **Skc** at levels approx. twice as high as the wild type.

L11 ANSWER 65 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:585755 HCAPLUS  
DOCUMENT NUMBER: 117:185755  
TITLE: High-level **expression** of degraded  
product-free **streptokinase** in Escherichia  
coli by removal of its putative leader sequence.  
[Erratum to document cited in CA116(13):122160m]  
AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon;  
Chun, Moon Jin; Byun, Si Myung  
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol.,  
Seoul, 130-650, S. Korea  
SOURCE: Molecules and Cells (1992), 2(1), 119  
CODEN: MOCEEK; ISSN: 1016-8478  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The omission of acknowledgment of a research grant has been corrected The  
error was not reflected in the abstract or the index entries.

L11 ANSWER 66 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1992-03808 BIOTECHDS  
TITLE: Method for the isolation and **expression** of a gene  
encoding **streptokinase**;  
Streptococcus **equisimilis** gene cloning  
and vector plasmid pEKG3, plasmid pPESKC-4 and plasmid  
pPISKC-6 **expression** in Escherichia coli or  
Pichia pastoris  
PATENT ASSIGNEE: Cent.Ing.Genet.Biotechnol.  
PATENT INFO: AU 9178101 28 Nov 1991  
APPLICATION INFO: AU 1991-78101 31 May 1991  
PRIORITY INFO: CU 1990-90 23 May 1990  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 1992-024716 [04]

AB A new method for the isolation and **expression** of a gene,  
SKC-2, encoding Streptococcus **equisimilis** C  
(ATCC 9542) **streptokinase** comprises (i) gene amplification from  
synthetic oligonucleotides SK1, SK2 and SK3 (specified DNA sequence),  
(ii) cloning SKC-2 in bacteria (preferably  
Escherichia coli) with or without a signal peptide; and (iii) intra- or  
extracellular **expression** in yeast (preferably Pichia pastoris),  
with the transformed microorganism displaying a high stability and level  
of **expression**. The following are also claimed: (1) plasmid  
pEKG3 containing SKC-2 inserted between the trp  
promoter and the phage T4 terminator for **expression** in  
bacteria; (2) plasmid pPESKC-4 and plasmid pPISKC-6, obtained by  
insertion of SKC-2 in the yeast **expression**  
vectors plasmid pPS-7 and plasmid pNAO, respectively, for extra- or  
intracellular **expression**; (3) transformed microorganisms  
displaying high levels of SKC-2 gene  
**expression**, good viability and cellular stability; (4) the  
product resulting from **expression** of the SKC-  
2 gene in bacteria and yeast; (5) recombinant DNA  
comprising the SKC-2 DNA sequence; and (6) the  
**expression** product of the recombinant DNA. (28pp)

L11 ANSWER 67 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:672660 HCAPLUS  
DOCUMENT NUMBER: 115:272660  
TITLE: Recombinant of thrombolytic and fibrinolytic  
enzymes as inactive dimers linked by sequence  
recognized by blood coagulation factors  
INVENTOR(S): Dawson, Keith Martyn; Hunter, Michael George;  
Czaplewski, Lloyd George  
PATENT ASSIGNEE(S): British Bio-Technology Ltd., UK  
SOURCE: PCT Int. Appl., 110 pp.

DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 4  
 PATENT INFORMATION:

CODEN: PIXXD2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9109125	A1	19910627	WO 1990-GB1911	19901207
W: AU, CA, FI, HU, JP, KR, NO, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
CA 2069085	AA	19910608	CA 1990-2069085	19901207
CA 2069085	C	20000201		
CA 2069105	AA	19910608	CA 1990-2069105	19901207
AU 9169540	A1	19910718	AU 1991-69540	19901207
AU 644399	B2	19931209		
ZA 9009853	A	19920826	ZA 1990-9853	19901207
ZA 9009854	A	19920826	ZA 1990-9854	19901207
EP 504241	A1	19920923	EP 1991-900869	19901207
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 155812	E	19970815	AT 1991-900851	19901207
ES 2106073	T3	19971101	ES 1991-900851	19901207
IL 96601	A1	19990509	IL 1990-96601	19901207
JP 05502374	T2	19930428	JP 1991-501314	19911115
JP 2900606	B2	19990602		
US 5434073	A	19950718	US 1992-854596	19920603
FI 9202609	A	19920605	FI 1992-2609	19920605
NO 9202237	A	19920806	NO 1992-2237	19920605
AU 9344976	A1	19931118	AU 1993-44976	19930830

PRIORITY APPLN. INFO.:  
 GB 1989-27722 A 19891207  
 WO 1990-GB1911 A 19901207

AB Fibrinolytic or thrombolytic enzymes are manufactured in a **recombinant** host as inactive fusion proteins containing two or more sequences of the protein linked by a peptide that can be cleaved by a blood-coagulation factor. The construction of **expression** vectors for the manufacture of hirudin or **streptokinase** dimers linked by peptides cleavable by Factor Xa or thrombin for *Escherichia coli* or *Saccharomyces cerevisiae* (with or without product secretion) is described. All the products tested were cleavable by the appropriate factors.

L11 ANSWER 68 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:443480 HCAPLUS

DOCUMENT NUMBER: 115:43480

TITLE: Synthetic genes for **streptokinase** and **streptokinase** analogs and their **expression** in *Escherichia coli*

INVENTOR(S): Fujii, Setsuro; Katano, Tamiki; Majima, Eiji; Ogino, Koichi; Ono, Kenji; Sakata, Yasuyo; Uenoyama, Tsutomu

PATENT ASSIGNEE(S): Otsuka Pharmaceutical Factory, Inc., Japan

SOURCE: Eur. Pat. Appl., 76 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 407942	A2	19910116	EP 1990-113099	19900709
EP 407942	A3	19910904		
EP 407942	B1	19951011		
R: AT, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
JP 04011892	A2	19920116	JP 1990-179851	19900706
US 5240845	A	19930831	US 1990-549049	19900706

AU 9058806	A1	19910117	AU 1990-58806	19900709
AU 648029	B2	19940414		
AT 129014	E	19951015	AT 1990-113099	19900709
ES 2078925	T3	19960101	ES 1990-113099	19900709
CA 2020828	AA	19910112	CA 1990-2020828	19900710
PRIORITY APPLN. INFO.:			JP 1989-179432	A 19890711
			JP 1989-307957	A 19891127
			JP 1990-96830	A 19900411

AB Genes encoding **streptokinase** (I) and its derivs. are synthesized and **expressed** in a host such as *Escherichia coli* for manufacture of I suitable for clin. application. The DNA encoding natural-type I was synthesized by standard chemical and used for construction of **expression** plasmid pSKXT, which in turn **expressed** the I gene using the *E. coli* tac promoter and the *blc* signal sequence. Efficient **expression** of the gene in the *E. coli* transformants and purification of the protein product were demonstrated. I analogs with a carboxy-terminal deletions, optionally with internal modifications were also described.

L11 ANSWER 69 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1991:513401 BIOSIS  
DOCUMENT NUMBER: PREV199141114116; BR41:114116  
TITLE: **EXPRESSION AND PROPERTIES OF HYBRID STREPTOKINASES EXTENDED BY AMINO-TERMINAL PLASMINOGEN KRINGLE DOMAINS.**  
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J  
CORPORATE SOURCE: DEP MICROBIOL IMMUNOL, UNIV OKLA HEALTH SCI CENTER, OKLAHOMA CITY, OKLA 73190, USA  
SOURCE: (1991) pp. 184-189. DUNNY, G. M., P. P. CLEARY AND L. L. MCKAY (ED.). GENETICS AND MOLECULAR BIOLOGY OF STREPTOCOCCI, LACTOCOCCI, AND ENTEROCOCCI; THIRD INTERNATIONAL ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, MINNEAPOLIS, MINNESOTA, USA, JUNE 6-9, 1990. VIII+310P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS.  
ISBN: 1-55581-034-9.  
DOCUMENT TYPE: Book  
Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 14 Nov 1991  
Last Updated on STN: 14 Nov 1991

L11 ANSWER 70 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:100297 HCAPLUS  
DOCUMENT NUMBER: 116:100297  
TITLE: Secretion of **streptokinase** from *Bacillus subtilis*  
AUTHOR(S): Kim, Sung Il; Lee, Se Yong; Byun, Si Myung  
CORPORATE SOURCE: Dep. Agric. Chem., Korea Univ., Seoul, 136-701, S. Korea  
SOURCE: Molecules and Cells (1991), 1(3), 325-31  
CODEN: MOCEEK; ISSN: 1016-8478  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB To secrete the **streptokinase** (SKC) from *B. subtilis*, three different **expression**-secretion vectors were constructed. One of them was pB140SK-I which contained the full **streptokinase** gene (*skc*) of *Streptococcus equisimilis* ATCC 9542 on pUB140 plasmid and the others were pB240SK-II and pB340SK-II which possessed the structural gene part of *skc* on secretion vectors pUBA240 and pUBS340, resp. PUBA240 and pUBS340 are secretion vectors constructed with  $\alpha$ -amylase promoter (*amyR2*) and signal sequence. When these **streptokinase** secretion vectors were transferred into

B. subtilis LKS86, streptokinase was expressed and secreted into the culture broth. Although own promoter and signal sequence of the *skc* gene functioned normally in B. subtilis, maximum amount of streptokinase secretion was obtained by LKS86 (pB240SK-II) system.

L11 ANSWER 71 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 27

ACCESSION NUMBER: 1991:96194 HCAPLUS

DOCUMENT NUMBER: 114:96194

TITLE: The leader sequence of streptokinase is responsible for its post-translational carboxyl-terminal cleavage

AUTHOR(S): Park, Seung Kook; Lee, Byeong Ryong; Byun, Si Myung

CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea

SOURCE: Biochemical and Biophysical Research Communications (1991), 174(1), 282-6

CODEN: BBRC9; ISSN: 0006-291X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB When the streptokinase gene from Streptococcus equisimillis was expressed from 2 tac promoter-controlled expression vectors, one deleted the putative leader sequence of streptokinase. Both normal and degraded streptokinase were detected in proteins expressed from the leader-encoding vector, but only normal streptokinase was detected from the leader-deleted vector. These findings indicate that the characteristic carboxyl-terminal cleavage of streptokinase is correlated with its leader sequence and occurs during defective secretion. A homogeneous preparation of streptokinase was facilitated by expression from this leader-deleted vector.

L11 ANSWER 72 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:122160 HCAPLUS

DOCUMENT NUMBER: 116:122160

TITLE: High-level expression of degraded product-free streptokinase in Escherichia coli by removal of its putative leader sequence

AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung

CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea

SOURCE: Molecules and Cells (1991), 1(2), 187-92

CODEN: MOCEEK; ISSN: 1016-8478

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The expression vector for streptokinase has been constructed from the previously cloned streptokinase -coding gene (*skc*) from Streptococcus equisimilis. Because of its deleterious effect on the host cell growth, the leader sequence of *skc* was removed and the leader sequence-deleted *skc* was subcloned into the vector pKK223-3, which contains the regulatable tac promoter and rrnB T1T2 transcription terminator, with a short synthetic oligonucleotide adapter. When this vector, pKS601 having *skc* gene, was expressed in E. coli, a 47.4-kDa protein was found to be newly accumulated to about 12% of the total cellular proteins, and it was identified as the streptokinase by immunoblotting with rabbit anti-streptokinase polyclonal serum. The expressed streptokinase was free from carboxyl-terminal degraded 44-kDa streptokinase and purified to near homogeneity using DEAE-cellulose and Sephadex G-150 columns. Its specific activity was about 1.3 + 105 CLN units/mg protein.

L11 ANSWER 73 OF 106

MEDLINE on STN

DUPLICATE 28

ACCESSION NUMBER: 92039051 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 1937032  
 TITLE: Isolation, sequence and **expression** in *Escherichia coli*, *Bacillus subtilis* and *Lactococcus lactis* of the DNase (streptodornase)-encoding gene from *Streptococcus equisimilis* H46A.  
 AUTHOR: Wolinowska R; Ceglowski P; Kok J; Venema G  
 CORPORATE SOURCE: Department of Pharmaceutical Microbiology, Medical Academy, Warsaw, Poland.  
 SOURCE: Gene, (1991 Sep 30) 106 (1) 115-9.  
 Journal code: 7706761. ISSN: 0378-1119.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-M59725; GENBANK-M59726; GENBANK-M59727;  
 GENBANK-M59728; GENBANK-M63990; GENBANK-S61507;  
 GENBANK-S63856; GENBANK-S63863; GENBANK-S65020;  
 GENBANK-S65060; GENBANK-X17241  
 ENTRY MONTH: 199112  
 ENTRY DATE: Entered STN: 19920124  
 Last Updated on STN: 19920124  
 Entered Medline: 19911223

AB A partial library of BclI-generated chromosomal DNA fragments from *Streptococcus equisimilis* H64A (Lancefield Group C) was constructed in *Escherichia coli*. Clones displaying either **streptokinase** or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdC) **expressing** the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdC gene was **expressed** in *E. coli*, *Bacillus subtilis* and *Lactococcus lactis*. As observed for *S. equisimilis*, in the heterologous Gram + hosts, at least part of the SDC protein was secreted into the medium.

L11 ANSWER 74 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
 DUPLICATE 29

ACCESSION NUMBER: 1991-08049 BIOTECHDS  
 TITLE: High yield production of **recombinant** serotype-C **streptokinase**;  
 gene cloning; **expression** in  
*Streptococcus equisimilis* containing 2 gene  
 copies and a selectable marker gene using vector plasmid  
 pSM752 or plasmid pSMS69

PATENT ASSIGNEE: Akad.Wiss.DDR  
 PATENT INFO: DD 284898 28 Nov 1990  
 APPLICATION INFO: DD 1989-332866 21 Sep 1989  
 PRIORITY INFO: DD 1989-332866 21 Sep 1989  
 DOCUMENT TYPE: Patent  
 LANGUAGE: German  
 OTHER SOURCE: WPI: 1991-125491 [18]

AB **Recombinant** serotype-C **streptokinase** (Skc)  
 production comprises cultivation of *Streptococcus equisimilis*  
 H464, C9/50 or SSIC type 20 transformants harboring a vector plasmid that  
 includes the Skc gene and a selectable marker (SM) gene as well  
 as **expression** and protein secretion signals. The transformant  
 is cultured for 8-20 generations without selection pressure, and strains  
 lacking plasmids but carrying the specified markers are then cultured for  
 Skc production. The SM gene preferably encodes resistance to  
 macrolide-lincosamide-streptogramin B (MLS), chloramphenicol, kanamycin  
 or streptomycin. The transformants contain 2 copies of the Skc  
 gene incorporated into their chromosomes by homologous recombination.



They produce **Skc** at a greater rate than e.g. H46A. The inherited marker is retained stably over many generations. The **Skc** gene is preferably present on a PstI fragment (transformation is then with plasmid pSM752) or a 2076 bp fragment (transformation with plasmid pSMS69); both vectors contain the MLS-resistance marker. Transformation is by electroporation or PEG-mediated protoplast transformation. (12pp)

L11 ANSWER 75 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1991-01878 BIOTECHDS

TITLE: Constructing vector for detecting **expression** of foreign genes;  
by inserting element containing **expression** unit, **streptokinase** gene and restriction sites, allowing in frame gene insertion; pro-chymosin, beta-galactosidase production

PATENT ASSIGNEE: Akad.Wiss.DDR

PATENT INFO: DD 279900 20 Jun 1990

APPLICATION INFO: DD 1987-306609 3 Sep 1987

PRIORITY INFO: DD 1987-306609 3 Sep 1987

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 1990-342373 [46]

AB Construction of vectors for detecting heterologous gene **expression** comprises: i. incorporating (in order from N terminus) into a cloning vector, a polylinker or restriction site (RS1); **expression** or **expression** secretion unit (Escherichia coli lac operon or exotoxin A gene of phage T12 from Streptococcus pyogenes; polylinker or restriction site (RS2); **streptokinase** (SK) structural gene (from Streptococcus equisimilis H46A, particularly a 1596 bp HindIII fragment from plasmid pMF5), a polylinker or restriction site (RS3); ii. inserting a foreign gene, X, without a promoter into polylinker or RS2; iii. the resulting detection vector, encoding for an X-SK fusion product, is used to transform microbial receptor cells; and iv. subjecting recombinant clones to a plasminogen-milk agar (PMA) overlaying test. Preferably, the vector is a bacterial plasmid or an M13 E. coli phage vector. X is a pro-chymosin gene (plasmid pHRW400 or plasmid pHRW500), human interferon-alpha-1, or beta-galactosidase (EC-3.2.1.23, from a pUC plasmid). Recipients are E. coli JM101, Streptococcus sanguis Challis 6 or Streptococcus lactis MG1363. (14pp)

L11 ANSWER 76 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:179754 HCAPLUS

DOCUMENT NUMBER: 114:179754

TITLE: Fusion proteins of **streptokinase** and human plasminogen

INVENTOR(S): Malke, Horst; Ferretti, Joseph J.

PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Zentralinstitut fuer Mikrobiologie und Experimentelle Therapie, Ger. Dem. Rep.

SOURCE: Ger. (East), 42 pp.

CODEN: GEXXA8

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 284484	A5	19901114	DD 1989-328631	19890516
PRIORITY APPLN. INFO.:			DD 1989-328631	19890516

AB A hybrid **streptokinase** is produced in prokaryotic cells. The **streptokinase**, which displays thrombin selectivity, consists of

the N-terminal kringle domains of human plasminogen fused to C-terminal *Streptococcus equisimilis* streptokinase. *Escherichia coli* transformed with plasmids encoding the described fusion protein fused to the N-terminal hexapeptide of  $\beta$ -galactosidase produced the hybrid **streptokinase** which was purified from cell lysates by immuno-affinity chromatog. and by chromatog. on lysine Sepharose.

L11 ANSWER 77 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:162447 HCAPLUS  
DOCUMENT NUMBER: 114:162447  
TITLE: **Recombinant manufacture of streptokinase**  
INVENTOR(S): Laplace, Frank; Mueller, Joerg; Malke, Horst  
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Patentabteilung, Ger. Dem. Rep.  
SOURCE: Ger. (East), 9 pp.  
CODEN: GEXXA8  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 282709	A5	19900919	DD 1988-323948	19881227
PRIORITY APPLN. INFO.:			DD 1988-323948	19881227

AB **Streptokinase** from *Streptococcus equisimilis* serotype C is manufactured by **expression** of the **skc** gene in *Escherichia coli*, *Bacillus subtilis*, or other *Streptococcus*. The natural **expression** cassette for the **skc** gene was introduced into a broad host-range vector to give plasmid pMLS10. Transformants of *Streptococcus sanguis* carrying this vector produced 750-1,000 **streptokinase** units/mL in a complex medium after 16 h growth at 36°, at this point the culture reached stationary phase and the enzyme continued to be slowly accumulated.

L11 ANSWER 78 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:141637 HCAPLUS  
DOCUMENT NUMBER: 114:141637  
TITLE: **Manufacture of streptokinase with bacterial L-forms**  
INVENTOR(S): Laplace, Frank; Mueller, Joerg; Gumpert, Johannes; Malke, Horst  
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Patentabteilung, Ger. Dem. Rep.  
SOURCE: Ger. (East), 7 pp.  
CODEN: GEXXA8  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 281816	A5	19900822	DD 1988-323947	19881227
PRIORITY APPLN. INFO.:			DD 1988-323947	19881227

AB **Streptokinase** is manufactured by **expression** of the **skc** gene from serotype C of *Streptococcus equisimilis* in the L-form of a bacterial host (*Bacillus* or *Proteus*). The construction of the small plasmid pMLS10 (6.7 kb) for **expression** of the gene in L-forms of *P. mirabilis* and *B. subtilis* is described.

L11 ANSWER 79 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN  
DUPLICATE 30

ACCESSION NUMBER: 90356604 EMBASE  
DOCUMENT NUMBER: 1990356604  
TITLE: Duplication of the **streptokinase** gene in the  
chromosome of *Streptococcus equisimilis* H46A.  
AUTHOR: Muller J.; Malke H.  
CORPORATE SOURCE: Central Inst. Microbiology, Beutenbergstrasse 11, Jena-6900,  
Germany  
SOURCE: FEMS Microbiology Letters, (1990) Vol. 72, No. 1-2, pp.  
75-78.  
ISSN: 0378-1097 CODEN: FMLED7  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 911213  
Last Updated on STN: 911213

AB The erythromycin resistance plasmid pSM752 carrying the **cloned streptokinase** gene, **skc**, was introduced by protoplast transformation into *Streptococcus equisimilis* H46A from which **skc** was originally **cloned**. Cells transiently supporting the replication of pSM752 gave rise to an erythromycin-resistant clone designated H46SM which was plasmid free and produced **streptokinase** at levels approximately twice as high as the wild type. Southern hybridization of total cell DNA with an **skc** -containing probe provided evidence for the duplication of the **skc** gene in the H46SM chromosome. The results, which have some bearing on industrial **streptokinase** production, can be best explained by a single cross-over event between the chromosome and the plasmid in the region of shared homology leading to the integration of pSM752 in a Campbell-like manner.

L11 ANSWER 80 OF 106 MEDLINE on STN  
ACCESSION NUMBER: 91130849 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 2283044  
TITLE: Duplication of the **streptokinase** gene in the  
chromosome of *Streptococcus equisimilis* H46A.  
AUTHOR: Muller J; Malke H  
CORPORATE SOURCE: Academy of Sciences of the G.D.R., Central Institute of  
Microbiology and Experimental Therapy, Jena.  
SOURCE: FEMS microbiology letters, (1990 Oct) 60 (1-2) 75-8.  
Journal code: 7705721. ISSN: 0378-1097.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199103  
ENTRY DATE: Entered STN: 19910405  
Last Updated on STN: 19970203  
Entered Medline: 19910321

AB The erythromycin resistance plasmid pSM752 carrying the **cloned streptokinase** gene, **skc**, was introduced by protoplast transformation into *Streptococcus equisimilis* H46A from which **skc** was originally **cloned**. Cells transiently supporting the replication of pSM752 gave rise to an erythromycin-resistant clone designated H46SM which was plasmid free and produced **streptokinase** at levels approximately twice as high as the wild type. Southern hybridization of total cell DNA with an **skc** -containing probe provided evidence for the duplication of the **skc** gene in the H46SM chromosome. The results, which have some bearing on industrial **streptokinase** production, can be best explained by a single cross-over event between the chromosome and the plasmid in the region of shared homology leading to the integration of pSM752 in a Campbell-like manner.

L11 ANSWER 81 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1989:206803 HCAPLUS

DOCUMENT NUMBER: 110:206803

TITLE: Streptokinase mutations relieving  
Escherichia coli K-12 (prlA4) of detriments caused by  
the wild-type skc gene

AUTHOR(S): Mueller, Joerg; Reinert, Hilmer; Malke, Horst

CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Therapy, Acad. Sci. G. D.  
R., Jena, 6900, Ger. Dem. Rep.

SOURCE: Journal of Bacteriology (1989), 171(4), 2202-8

CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A novel phenotype is described for E. coli K-12 carrying the prlA4 allele determining a membrane component of the protein export mechanism. It is manifest as transformation deficiency for plasmids containing the cloned group C streptococcal streptokinase gene, skc. Streptokinase plasmid mutations relieving the prlA4 strain of this deficiency fell into three classes. Class 1 included skc::IS5 insertions, with IS5 integrated in a region encoding the Skc signal sequence and inactivating skc. Class 2 included IS1 insertions leaving skc intact but reducing skc expression, presumably by altering the function of the skc promoter as judged by an insertion site close to the -35 region. Class 3 included skc deletions removing the entire signal sequence or a tetrapeptide from its hydrophobic core. The tetrapeptide deletion reduced the size, hydrophobicity, and predicted  $\alpha$ -helicity of the central region of the Skc signal sequence but facilitated the export of mature Skc in both the wild type and the prlA4 mutant. These findings indicate that the incompatibility between prlA4 and skc is related to deleterious effects of the Skc signal sequence. The tetrapeptide deletion may function by altering the conformation of the signal sequence so as to render interaction with both the PrlA wild-type protein and the PrlA4 mutant protein less detrimental to the export mechanism. These findings also provide an explanation for the difficulties encountered in cloning streptokinase genes in E. coli plasmids and maintaining their structural stability.

L11 ANSWER 82 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1990-02600 BIOTECHDS

TITLE: Site-specific alteration of Gly-24 in streptokinase  
: its effect on plasminogen activation;  
site-directed mutagenesis effect on plasminogen-activator  
activity; gene cloning and expression  
in Escherichia coli

AUTHOR: Lee B R; Park S K; Kim J H; \*Byun S M

LOCATION: Department of Biological Science and Engineering, Korea  
Advanced Institute of Science and Technology (KAIST), P.O.  
Box 150, Cheongryang, Seoul, Korea.

SOURCE: Biochem.Biophys.Res.Commun.; (1989) 165, 3, 1085-90

CODEN: BBRC A9

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Oligonucleotide site-directed mutagenesis was performed to replace Gly-24 of Streptococcus equisimilis (ATCC 9542) streptokinase with His, Glu, or Ala. The streptokinase gene was cloned, subjected to mutagenesis for removal of the RsaI site, cloned into vector plasmid pKS601 under the control of the trp promoter and used to transform Escherichia coli C600. The recombinant proteins were purified by DEAE-cellulose and Sephadex-G150 chromatography. Substitutions with either His or Glu gave almost complete loss of streptokinase activity but

streptokinase replaced with Ala retained its activity. Although streptokinases with His-24 or Glu-24 bound normally to human plasminogen, they did not generate active plasmin, whereas those with Ala-24 or Gly-24 generated active plasmin. The results indicate that the small, uncharged alkyl group side chain on the 24th amino acid residue of streptokinase is indispensable for the activity of the human plasminogen-streptokinase complex. A charged amino acid in position 24 disrupts beta-sheet formation and prevents streptokinase from adopting the orientation required for plasminogen activation. (26 ref)

L11 ANSWER 83 OF 106 MEDLINE on STN DUPLICATE 31  
 ACCESSION NUMBER: 90172183 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 2625666  
 TITLE: Sequence-directed DNA bending upstream of the streptokinase promoter.  
 AUTHOR: Muller J; Malke H  
 CORPORATE SOURCE: Akademie der Wissenschaften der DDR.  
 SOURCE: Journal of basic microbiology, (1989) 29 (9) 611-6.  
 Journal code: 8503885. ISSN: 0233-111X.  
 PUB. COUNTRY: GERMANY, EAST: German Democratic Republic  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199004  
 ENTRY DATE: Entered STN: 19900601  
 Last Updated on STN: 19900601  
 Entered Medline: 19900409

AB A 450-base pair (bp) HinfI restriction fragment from the chromosome of Streptococcus equisimilis H46A contains the early coding region of the streptokinase gene (skc), the skc promoter, and a stretch of DNA 5' to the--35 region of the skc promoter. Two-dimensional polyacrylamide (PA) gel electrophoresis at two different temperatures showed that this fragment migrates anomalously slowly on PA gels, suggesting the existence of a bent DNA conformation. Inspection of the nucleotide sequence confirmed this suggestion by revealing numerous oligomeric dA.dT tracts, some of which are in phase with the helix screw. Computer analysis of the sequence predicted the existence of two bending loci, one of which is located upstream of the skc promoter. In addition to showing DNA bending, the 450-bp HinfI fragment contains multiple 13-bp sequences homologous to the Escherichia coli integration host factor DNA-binding consensus sequence. Insertion of IS1 into a site immediately upstream of the--35 region decreased the expression level of skc in E. coli, suggesting that DNA conformation upstream of the promoter has a role in skc expression.

L11 ANSWER 84 OF 106 MEDLINE on STN DUPLICATE 32  
 ACCESSION NUMBER: 88302119 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 3043172  
 TITLE: Tripartite streptokinase gene fusion vectors for gram-positive and gram-negative procaryotes.  
 AUTHOR: Klessen C; Schmidt K H; Ferretti J J; Malke H  
 CORPORATE SOURCE: Academy of Sciences of the GDR, Central Institute of Microbiology and Experimental Therapy, Jena.  
 SOURCE: Molecular & general genetics : MGG, (1988 May) 212 (2) 295-300.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 198809  
 ENTRY DATE: Entered STN: 19900308

Last Updated on STN: 19900308

Entered Medline: 19880921

AB A specific 1,596 bp HincII fragment ('**skc**) from the chromosome of *Streptococcus equisimilis* contains an active **streptokinase** (SK) gene (**skc**) lacking, in addition to the **expression** signals, codons 1 through 39 of wild-type **skc** but retaining the remainder of the **skc** coding sequence together with the transcription terminator. Using this fragment as an indicator gene, we constructed two types of vectors which in appropriate hosts resulted in the synthesis of SK fusion proteins after insertional activation of '**skc**'. The first type are open reading frame (ORF) vectors in which '**skc** was inserted into pUC18 out of frame with respect to lacZ', thus conferring an SK-negative phenotype. Any DNA fragments representing ORFs inserted between the lacZ' **expression** signals and '**skc** such that the **skc** reading frame was restored resulted in the production of tripartite proteins which exhibited SK activity. The second type of vector, which functioned in both gram-positive and gram-negative bacteria, used the streptococcal **speA expression** and secretion signals in front of the ORF to activate '**skc** insertionally. Using a large fragment from the chymosin gene as the target sequence, the usefulness of these vectors for studying foreign gene **expression** in streptococci as well as *Escherichia coli* was demonstrated.

L11 ANSWER 85 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1988-10064 BIOTECHDS

TITLE: **Expression** of heterologous genes in *Pseudomonas putida* under the control of streptococcal promoters; interferon-alpha-1 and **streptokinase** gene **expression**

AUTHOR: Laplace F; Mueller J; Wollweber L; Malke H

LOCATION: Central Institute of Microbiology and Experimental Therapy, Beutenbergstrasse 11, Jena 6900, DDR.

SOURCE: FEMS Microbiol.Lett.; (1988) 52, 275-78

CODEN: FMLED7

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Expression** plasmids were constructed for *Pseudomonas putida* KT2440 using streptococcal promoters. The **speA** promoter/interferon-alpha1 (IFN-alpha1) cassette from plasmid pJC127 was inserted in plasmid pBR322, and joined with RSF1010 to form plasmid pJP127. The **streptokinase** (SK) gene (**skc**) and promoter from plasmid pMF5 were inserted in plasmid pUC19 and fused with RSF1010 to form plasmid pLMM192. **Skc** was **expressed** in *P. putida* with pLMM192, reaching maximum SK yields in early stationary phase (0.2-0.4 ug/ml culture). Most activity was distributed equally between periplasm and cytoplasm, with 2-5% in the supernatant. SDS-PAGE and protease treatment showed the *P. putida* protein was identical to those of *Streptococcus equisimilis* H46A and *Streptococcus sanguis* Challis. *P. putida* with pJP127 produced 100 000 U/l interferon (determined by radial immunodiffusion and ELISA). Immunodiffusion showed the protein to be identical with human IFN-alpha1. Antiviral assays showed that 70% activity was cytoplasmic, the remainder being periplasmic. Thus neither secretion vector mediated complete export into the periplasm. (29 ref)

L11 ANSWER 86 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
DUPLICATE 33

ACCESSION NUMBER: 1988-02891 BIOTECHDS

TITLE: **recombinant streptokinase** production in yeasts; plasmid vector cloning in *Pichia* sp. for thrombolytic enzyme preparation

PATENT ASSIGNEE: Phillips-Petrol.

PATENT INFO: EP 248227 9 Dec 1987  
APPLICATION INFO: EP 1987-106614 7 May 1987  
PRIORITY INFO: US 1986-960 8 May 1986  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 1987-343212 [49]

AB The production of **recombinant streptokinase** for use in thrombolytic enzyme preparations is new. A novel DNA sequence comprises: a yeast regulatory region which is capable of controlling transcription of mRNA in yeast when positioned at the 5'-end of a polypeptide encoding region; and a polypeptide coding region encoding **streptokinase** or portions thereof free from bacterial signal sequence. The regulatory region (RR) is preferably selected from the Pichia AOX1 RR, DAS1 RR, Pichia glyceraldehyde-3-phosphate-dehydrogenase RR, Saccharomyces cerevisiae acid phosphatase RR, Saccharomyces alpha-mating factor RR, Saccharomyces glyceraldehyde-5-phosphate-dehydrogenase RR and the Pich p40 RR. Also new is the DNA sequence contained in a vector, plasmid pHTskc25, along with a selectable marker gene and a yeast autonomous replication sequence. **Streptokinase** may be produced at high yields by culturing Pichia sp. transformed with plasmid pHTskc25. The **streptokinase** gene is from Streptococcus equisimilis. (27pp)

L11 ANSWER 87 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1987:499919 BIOSIS  
DOCUMENT NUMBER: PREV198733127633; BR33:127633  
TITLE: STREPTOKINASE EXPRESSION OF ALTERED FORMS.  
AUTHOR(S): MALKE H [Reprint author]; LORENZ D; FERRETTI J J  
CORPORATE SOURCE: ACAD SCI GER DEMOCRATIC REPUBLIC, CENT INST MICROBIOL AND EXP THERAPY, DDR-69 JENA, GDR  
SOURCE: (1987) pp. 143-149. FERRETTI, J. J. AND R. CURTISS, III (ED.). STREPTOCOCCAL GENETICS; SECOND ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, MIAMI, FLORIDA, USA, MAY 21-24, 1986. VIII+300P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS.  
ISBN: 0-914826-93-X.  
DOCUMENT TYPE: Book  
Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 27 Nov 1987  
Last Updated on STN: 27 Nov 1987

L11 ANSWER 88 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1988:487410 HCAPLUS  
DOCUMENT NUMBER: 109:87410  
TITLE: Methylo-trophic yeast as vehicles for heterologous gene **expression**  
AUTHOR(S): Stroman, D. W.; Hagenson, M. J.  
CORPORATE SOURCE: Phillips Res. Cent., Phillips Pet. Co., OK, USA  
SOURCE: DECHEMA Monographien (1987), 105(Physiol. Genet. Modulation Prod. Form.), 141-6  
CODEN: DMDGAG; ISSN: 0070-315X  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The methylo-trophic yeast, Pichia pastoris, has been developed as a superior **recombinant** DNA (rDNA) production host. The key component in the development of this host was the **cloning** of the alc. oxidase gene and use of its promoter-regulatory region to control gene **expression**. Heterologous **expression** of several foreign genes in this yeast has been studied. The promoter-regulatory region from the alc. oxidase gene permits very high per cell levels of gene

expression in an easily regulated manner. These high per cell levels of expression can be combined with high cell d. fermentation technol. to yield very high per L production of rDNA products. This is shown by the high levels of production of streptokinase in this yeast.

L11 ANSWER 89 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:455636 HCAPLUS  
DOCUMENT NUMBER: 105:55636  
TITLE: The streptokinase gene: cloning, sequencing and expression in new hosts  
AUTHOR(S): Malke, Horst  
CORPORATE SOURCE: Zentralinst. Mikrobiol., Dtsch. Akad. Wiss., Jena, Ger. Dem. Rep.  
SOURCE: Zeitschrift fuer Klinische Medizin (1985) (1986), 41(7), 502-4  
CODEN: ZKMEEF; ISSN: 0233-1608  
DOCUMENT TYPE: Journal  
LANGUAGE: German

AB The streptokinase (I) [9002-01-1] gene (skc) of Streptococcus equisimilis H46A was cloned in Escherichia coli using vector  $\lambda$ L47. One of the recombinant clones was used to subclone skc in E. coli plasmid vectors. Plasmids pMF2 (10.4 kilobases, composed of pACYC184 plus a 6.4-kilobase EcoRI fragment) and pMF5 (6.9 kilobases, with a 2.5-kilobase fragment in the PstI site of pBR322) determined I formation in E. coli; expression of skc was independent of its orientation, indicating that the complete gene, together with its control elements, was present. The 2.5-kilobase PstI fragment of pMF5 was isolated and sequenced in the M13 system. Of 2568 base pairs, the largest open reading frame consisted of 1320 base pairs coding for prestreptokinase, corresponding to I plus its 26-amino acid leader sequence. Expression of skc was attained in S. sanguis after transformation with the shuttle vector pSM752. In fermentation expts., I production rates of 1500 U/mL were attained, which was below the levels obtained with S. equisimilis. Use of pSM752 for similar transformation of Bacillus subtilis is briefly discussed.

L11 ANSWER 90 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 34

ACCESSION NUMBER: 1987:277792 BIOSIS  
DOCUMENT NUMBER: PREV198784018831; BA84:18831  
TITLE: MOLECULAR CLONING OF STREPTOKINASE GENE FROM STREPTOCOCCUS-EQUISIMILIS AND ITS EXPRESSION IN ESCHERICHIA-COLI.  
AUTHOR(S): ROH D C [Reprint author]; KIM J H; PARK S K; LEE J W; BYRUN S M  
CORPORATE SOURCE: DEP BIOLOGICAL SCIENCE AND ENGINEERING, KOREA ADVANCED INST SCIENCE AND TECHNOLOGY KAIST, PO BOX 150 CHONGRYANG, SEOUL 131, KOREA  
SOURCE: Korean Biochemical Journal, (1986) Vol. 19, No. 4, pp. 391-398.  
CODEN: KBCJAK. ISSN: 0368-4881.  
DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 19 Jun 1987  
Last Updated on STN: 19 Jun 1987

AB The streptococcal genomic DNA digested with Pst I was cloned in E. coli HB101. The overlay technique of casein/plasminogen was used to screen the clones for recombinants carrying the streptokinase gene. The insert size of the plasmid carrying the streptokinase gene was a 2.5, 4.3, and 5.8 Kb, respectively. The restriction maps of all three hybrid plasmids were constructed by



digestion with Pst I, Pvu II, Sal I, Hind III, Ava I, BamH I, and Cla I. For the identification of cloned gene, **streptokinase** was highly purified from *S. equisimilis* by the methods of gel chromatography and isoelectric focusing and rabbits were immunized with this purified **streptokinase**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established that the enzyme from *E. coli* was identical to that from *S. equisimilis*. In the *E. coli* cell culture, we found the activity of **streptokinase** in all three principal locations of the cell. More than 50% were existed in the intracellular space.

L11 ANSWER 91 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN  
 ACCESSION NUMBER: 1986:509637 HCAPLUS  
 DOCUMENT NUMBER: 105:109637  
 TITLE: The **streptokinase** gene  
 AUTHOR(S): Malke, H.; Ferretti, J. J.  
 CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Ger. Acad. Sci., Jena, DDR-6900, Ger. Dem. Rep.  
 SOURCE: Folia Haematologica (Leipzig) (1986), 113(1-2), 88-98  
 CODEN: FOHEAW; ISSN: 0323-4347  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB *Streptococcus equisimilis* Gene **skc** for **streptokinase** [9002-01-1] was cloned on the vector L47 and then subcloned into plasmids pACYC184 and pBR322 to form recombinant plasmids pMF2 (10.4 kb) and pMF5 (9.9 kb), resp., for expression in *Escherichia coli*. Plasmid pMF5 contained a 2568-base-pair (bp) insert that included the 1320-bp coding sequence for prestreptokinase. The prestreptokinase comprised 440 amino acid residues, including a 26-amino acid signal peptide. The insert also contained the **skc** upstream regions involved in the regulation of transcription and translation and a 15-bp repeat located 34 bp downstream of the **skc** translation stop signal, which very likely represents the rho-independent transcription terminator. The **skc** gene showed no extended regions homologous to the staphylokinase gene. Heterologous **skc** gene expression was also attained in *S. sanguis* after subcloning of the gene of pMF5 onto plasmid pSM7 to form the bifunctional shuttle plasmid pSM752 (13.3 kb). Plasmid pSM752 was not only functional in *E. coli* and *S. sanguis*, but also in *Bacillus subtilis*. The cloned **streptokinase** expressed in *E. coli*, *S. sanguis*, or *B. subtilis* has the same specificity as that of the donor strain.

L11 ANSWER 92 OF 106 MEDLINE on STN DUPLICATE 35  
 ACCESSION NUMBER: 86281080 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 3090231  
 TITLE: Expression of the **streptokinase** gene from *Streptococcus equisimilis* in *Bacillus subtilis*.  
 AUTHOR: Klessen C; Malke H  
 SOURCE: Journal of basic microbiology, (1986) 26 (2) 75-81.  
 Journal code: 8503885. ISSN: 0233-111X.  
 PUB. COUNTRY: GERMANY, EAST: German Democratic Republic  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 198609  
 ENTRY DATE: Entered STN: 19900321  
 Last Updated on STN: 19900321  
 Entered Medline: 19860916

AB The previously cloned and sequenced **streptokinase** gene (**skc**) from *Streptococcus equisimilis* H46A was inserted into plasmid vectors capable of replication in *Bacillus subtilis*. The **skc** gene was expressed by use of its own transcription

and translation signals which appeared to meet the stringent requirements of *B. subtilis* for efficient foreign gene **expression**. The secreted **streptokinase** activity began to decline toward the end of the exponential growth phase suggesting that *B. subtilis* exoproteases hydrolyzed and inactivated the foreign protein.

L11 ANSWER 93 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1985-11044 BIOTECHDS

TITLE: New plasmid transformed strain of *Streptococcus sanguis*;  
production of a new form of **streptokinase** for the  
treatment of thromboembolic disease

PATENT ASSIGNEE: Arzneimittelwerke AG, Dresden

PATENT INFO: EP 150424 7 Aug 1985

APPLICATION INFO: EP 1984-115655 17 Dec 1984

PRIORITY INFO: DD 1983-258031 16 Dec 1983

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 1985-191492 [32]

AB A new **streptokinase** producing strain of *Streptococcus sanguis* (Challis) (pSM 752) is claimed. This has been transformed with a **streptokinase** gene inserted from the donor strain of *Streptococcus equisimilis* H46A. Also new is a uniform monogenetic **streptokinase** of mol. weight 42,000 and isoelectric point of 5-5.5, formed by the cultivation of the new strain or other *Streptococcus* sp. H strains transformed with plasmid pSM 752. The new strain is cultivated in a liquid nutrient medium at 25-40 deg, preferably 30 deg at pH 6-8, preferably 7.2 for 10-36 hr, preferably 24 hr. The sugar content of the medium is maintained at 0.2-2, preferably 1% and the nitrogen content is 0.1-0.8%, preferably 0.2-0.4%. The **streptokinase** is useful for the treatment of thromboembolic vascular disorders and, since it is formed without contamination by streptococcal toxins, extensive purification is not required. (11pp)

L11 ANSWER 94 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1985-08513 BIOTECHDS

TITLE: Production of **streptokinase**;  
by cultivation of *Escherichia coli* ATCC 39613 containing  
**recombinant** plasmid PMF1

PATENT ASSIGNEE: Phillips-Petrol.

PATENT INFO: AU 8433859 18 Apr 1985

APPLICATION INFO: AU 1984-33859 5 Oct 1984

PRIORITY INFO: US 1984-585417 2 Mar 1984; DD 1983-255523 10 Oct 1983

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1985-135032 [23]

AB A **recombinant** vector plasmid PMF1 for the transformation of a host to produce **streptokinase** is new. The vector contains a polydeoxyribonucleotide fragment insert which codes for the synthesis and secretion of **streptokinase**. The transformant microorganism is preferably *Escherichia coli* HB101 and the vector, plasmid pBR322. The fragment coding for **streptokinase** synthesis and secretion is derived from a microorganism of the genus *Streptococcus*, especially *Streptococcus equisimilis* strain H46A and may have restriction endonuclease cleavage sites at the termini e.g. it has 7400 bp and the cleavage sites are for HindIII. The **recombinant** vector is obtained by digestion of a vector with a restriction endonuclease to give linear DNA. This DNA is ligated to the **streptokinase** fragment to give the **recombinant**. This fragment is obtained by digestion of *Streptococcus equisimilis* with the same restriction endonuclease, especially Pst I, as is used to digest the initial vector. **Streptokinase** can be produced and isolated for use as a thrombolytic agent to facilitate the in vivo lysis or dissolution of blood clots. (28pp)

L11 ANSWER 95 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1985:608113 HCAPLUS  
DOCUMENT NUMBER: 103:208113  
TITLE: Streptokinase-coding recombinant  
vectors  
INVENTOR(S): Ferretti, Joseph J.; Malke, Horst  
PATENT ASSIGNEE(S): Phillips Petroleum Co. , USA  
SOURCE: Eur. Pat. Appl., 21 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 151337	A2	19850814	EP 1984-306851	19841008
EP 151337	A3	19861008		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
DD 249037	A1	19870826	DD 1983-255523	19831010
US 4764469	A	19880816	US 1984-585417	19840302
AU 8433859	A1	19850418	AU 1984-33859	19841005
AU 561372	B2	19870507		
ZA 8407873	A	19850529	ZA 1984-7873	19841008
AT 61816	E	19910415	AT 1984-306851	19841008
FI 8403963	A	19850411	FI 1984-3963	19841009
NO 8404039	A	19850411	NO 1984-4039	19841009
DK 8404822	A	19850426	DK 1984-4822	19841009
JP 60237995	A2	19851126	JP 1984-212403	19841009
ES 536623	A1	19870116	ES 1984-536623	19841009
CA 1223223	A1	19870623	CA 1984-464939	19841009
DD 273284	A5	19891108	DD 1984-268254	19841010
US 5066589	A	19911119	US 1988-212254	19880627
US 5187098	A	19930216	US 1992-888420	19920522
PRIORITY APPLN. INFO.:				DD 1983-255523 A 19831010
				US 1984-585417 A 19840302
				EP 1984-306851 A 19841008
				US 1988-212254 A2 19880627
				US 1989-348206 B1 19890509

AB Recombinant vectors that code for streptokinase [9002-01-1] are constructed and cloned in Escherichia coli. Thus, DNA from Streptococcus equisimilis was isolated and digested with the restriction endonuclease Sau3A. DNA fragments of between 4-15 kb were cloned into phage  $\lambda$  L47. The ligated phage was infectively added to E. coli lawns and streptokinase-producing clones were isolated. The DNA from one such clone,  $\lambda$  L47E skc was partially digested with HindIII and then inserted into the HindIII site of plasmid pBR322. The recombinant plasmids were used to transform E. coli strain HB101. The plasmid isolated from 1 streptokinase-producing strain, pMF1, was isolated and a restriction map was prepared. A nucleotide sequence anal. of pMF1 showed that the cloned fragment encoded for streptokinase as well as an amino-terminal signal peptide which is bound to streptokinase and which is hydrolyzed during a streptokinase secretion event.

L11 ANSWER 96 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1987:472094 HCAPLUS  
DOCUMENT NUMBER: 107:72094  
TITLE: New cloning vectors for Escherichia coli and Bacillus subtilis  
INVENTOR(S): Klessen, Christian; Malke, Horst  
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Ger. Dem. Rep.  
SOURCE: Ger. (East), 7 pp.

CODEN: GEXXA8  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 231083	A1	19851218	DD 1984-264255	19840619
PRIORITY APPLN. INFO.:			DD 1984-264255	19840619

AB New cloning vectors for *Escherichia coli* and *Bacillus subtilis*, derived from the bifunctional plasmid pGR71 by insertion of a promoter-containing DNA fragment into the unique HindIII site of pGR71 upstream of the chloramphenicol acetyltransferase gene, are described. The new plasmids pSM1711, pSM7711, and pSM7712 contain a *Streptococcus* promoter from plasmids pMF1, pSM10, or pSM7.

L11 ANSWER 97 OF 106 MEDLINE on STN DUPLICATE 36

ACCESSION NUMBER: 85232082 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 2989113  
TITLE: Nucleotide sequence of the *streptokinase* gene from *Streptococcus equisimilis* H46A.  
AUTHOR: Malke H; Roe B; Ferretti J J  
CONTRACT NUMBER: AI 9304 (NIAID)  
SOURCE: Gene, (1985) 34 (2-3) 357-62.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-K02986  
ENTRY MONTH: 198508  
ENTRY DATE: Entered STN: 19900320  
Last Updated on STN: 20000303  
Entered Medline: 19850821

AB The entire nucleotide sequence of a cloned 2568-bp PstI fragment from the genome of *Streptococcus equisimilis* H46A encoding the *streptokinase* gene (*skc*) has been determined. The longest open reading frame comprises 1320 bp which code for *streptokinase*. The protein is synthesized with a 26-amino acid residue N-terminal extension having properties characteristic of a signal peptide. Comparison of the deduced amino acid sequence with the available amino acid sequence of a commercial *streptokinase* reveals minor primary structure differences. The nucleotide sequencing of *skc* does not support the hypothesis that the gene has evolved by duplication and fusion, as suggested by internal twofold amino acid homologies of its product. Furthermore, the *skc* gene sequence shows no extended regions homologous to the staphylokinase gene. Upstream from the *skc* gene, the putative *skc* promoter and the ribosome-binding site sequence have been identified; downstream from the coding region, inverted repeat sequences thought to function as transcription terminators have been detected.

L11 ANSWER 98 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:473464 HCAPLUS  
DOCUMENT NUMBER: 105:73464  
TITLE: Hybridization of a cloned group C streptococcal *streptokinase* gene with DNA from other streptococcal species  
AUTHOR(S): Huang, T. T.; Malke, H.; Ferretti, J. J.  
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA  
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis.,

9th (1985), Meeting Date 1984, 234-6. Editor(s):  
Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi.  
Reedbooks: Bracknell, UK.  
CODEN: 55BSAN

DOCUMENT TYPE: Conference  
LANGUAGE: English

AB The previously cloned **streptokinase** [9002-01-1] gene (**skc**) of *Streptococcus equisimilis* and 2 subfragments were used as DNA hybridization probes to determine sequence homologies with other streptococcal species. The human pathogenic streptococci of strains A, C, and G were the only strains that had a pos. correlation between the ability to produce **streptokinase** and to hybridize with the gene **skc** DNA probe. In conjunction with other streptococcal DNA probes, such as streptolysin O, hyaluronidase, DNase, and erythrogenic toxins, the **skc** probe may be of diagnostic significance in the rapid identification of human pathogenic streptococci.

L11 ANSWER 99 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:473709 HCAPLUS

DOCUMENT NUMBER: 105:73709

TITLE: Cloning of streptococcal genes with  
*Streptococcus-Escherichia coli* shuttle vector pSA3

AUTHOR(S): Dao, M. L.; Ferretti, J. J.

CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK,  
USA

SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc.  
Lancefield Int. Symp. Streptococci Streptococcal Dis.,  
9th (1985), Meeting Date 1984, 233-4. Editor(s):  
Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi.  
Reedbooks: Bracknell, UK.  
CODEN: 55BSAN

DOCUMENT TYPE: Conference

LANGUAGE: English

AB A shuttle vector, the chimeric plasmid pSA3, which can replicate in both *E. coli* and *S. sanguis*, was constructed. Chromosomal DNA from *S. mutans* was ligated into this plasmid and cloned in *E. coli*. Of 472 clones tested, 43 clones expressed *S. mutans* surface antigens. A cloned *S. equisimilis* **streptokinase** [9002-01-1] gene was inserted into plasmid pSA3 and then used to transform *E. coli*, *S. sanguis*, and *S. mutans*, all of which expressed the cloned **streptokinase** gene.

L11 ANSWER 100 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:473707 HCAPLUS

DOCUMENT NUMBER: 105:73707

TITLE: Cloned **streptokinase** gene from  
*Streptococcus equisimilis* H46A

AUTHOR(S): Malke, H.; Ferretti, J. J.

CORPORATE SOURCE: Ger. Acad. Sci., Jena, Ger. Dem. Rep.

SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc.  
Lancefield Int. Symp. Streptococci Streptococcal Dis.,  
9th (1985), Meeting Date 1984, 221-2. Editor(s):  
Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi.  
Reedbooks: Bracknell, UK.  
CODEN: 55BSAN

DOCUMENT TYPE: Conference

LANGUAGE: English

AB The **streptokinase** [9002-01-1] gene **skc** of *S. equisimilis* was cloned in *Escherichia coli* with plasmid pBR322. Expression of gene **skc** was observed with both orientations of the gene, which indicated that its own promoter was present and was functional in *E. coli*. **Streptokinase** was excreted by the *E. coli* host. The gene contained a 1320-base-pair open reading frame which encodes 440 amino acids, including a signal peptide of

26 amino acids.

L11 ANSWER 101 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 1985:107414 HCAPLUS  
DOCUMENT NUMBER: 102:107414  
TITLE: Streptococcus-Escherichia coli shuttle vector pSA3 and  
its use in the cloning of streptococcal  
genes  
AUTHOR(S): Dao, My Lien; Ferretti, Joseph J.  
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK,  
73190, USA  
SOURCE: Applied and Environmental Microbiology. (1985), 49(1),  
115-19  
CODEN: AEMIDF; ISSN: 0099-2240  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB A shuttle vector that can replicate in both Streptococcus and E. coli was constructed by joining the E. coli plasmid pACYC184 (chloramphenicol [56-75-7] and tetracycline [60-54-8] resistance) to the streptococcal plasmid pGB305 ( erythromycin [114-07-8] resistance). The resulting chimeric plasmid is designated pSA3 (chloramphenicol, erythromycin, and tetracycline resistance) and had 7 unique restriction sites: EcoRI, EcoRV, BamHI, SalI, XbaI, NruI, and SphI. Mol. cloning into the EcoRI or EcoRV site results in inactivation of chloramphenicol resistance, and cloning into the BamHI, SalI site results in inactivation of tetracycline resistance in E. coli. Plasmid pSA3 was transformed and was stable in S. sanguis and S. mutans in the presence of erythromycin. Plasmid pSA3 was used to construct a library of the S. mutans GS5 genome in E. coli, and expression of surface antigens in this heterologous host was confirmed with S. mutans antiserum. A previously cloned determinant that species streptokinase [9002-01-1] was subcloned into pSA3, and this recombinant plasmid was stable in the presence of a selective pressure and expressed streptokinase activity in E. coli, S. sanguis, and S. mutans.

L11 ANSWER 102 OF 106 MEDLINE on STN DUPLICATE 37  
ACCESSION NUMBER: 84221999 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 6374659  
TITLE: Streptokinase: cloning,  
expression, and excretion by Escherichia coli.  
AUTHOR: Malke H; Ferretti J J  
CONTRACT NUMBER: AI 9304 (NIAID)  
SOURCE: Proceedings of the National Academy of Sciences of the  
United States of America, (1984 Jun) 81 (11) 3557-61.  
Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198407  
ENTRY DATE: Entered STN: 19900320  
Last Updated on STN: 19970203  
Entered Medline: 19840724

AB Genomic DNA from Streptococcus equisimilis strain H46A was cloned in Escherichia coli by using the bacteriophage lambda replacement vector L47 and an in vitro packaging system. A casein/plasminogen overlay technique was used to screen the phage bank for recombinants carrying the streptokinase gene ( skc ). The gene was present with a frequency of 1 in 836 recombinants, and 10 independent clones containing skc were isolated and physically characterized. One recombinant clone was used to subclone skc in E. coli plasmid vectors. Plasmid pMF2 [10.4 kilobases (kb)] consisting of

pACYC184 with a 6.4-kb H46A DNA fragment in the EcoRI site and pMF5 (6.9 kb) carrying a 2.5-kb fragment in the Pst I site of pBR322 were among the recombinant plasmids determining streptokinase production in three different E. coli host strains. Expression of skc was independent of its orientation in either vector, indicating that its own promoter was present and functional in E. coli. However, expression in pBR322 was more efficient in one orientation than in the other, suggesting that one or both of the bla gene promoters contributed to skc expression. Several lines of evidence, including proof obtained by the immunodiffusion technique, established the identity of E. coli streptokinase. Testing cell-free culture supernatant fluids, osmotic shock fluids, and sonicates of osmotically shocked cells for streptokinase activity revealed the substance to be present in all three principal locations, indicating that E. coli cells were capable of releasing substantial amounts of streptokinase into the culture medium.

L11 ANSWER 103 OF 106 MEDLINE on STN DUPLICATE 38  
 ACCESSION NUMBER: 85035880 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 6593564  
 TITLE: Expression of a streptokinase gene from Streptococcus equisimilis in Streptococcus sanguis.  
 AUTHOR: Malke H; Gerlach D; Kohler W; Ferretti J J  
 SOURCE: Molecular & general genetics : MGG, (1984) 196 (2) 360-3. Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 198412  
 ENTRY DATE: Entered STN: 19900320  
 Last Updated on STN: 19900320  
 Entered Medline: 19841218

AB Using recombinant DNA techniques, we introduced a previously cloned streptokinase gene from Streptococcus equisimilis into the Challis strain of S. sanguis (group H). The gene was expressed in the new host under the control of its own promoter and the gene product had biological properties identical to authentic streptokinase. However, the molecular weight of cloned streptokinase (42 K) as expressed by S. sanguis was substantially lower than that of authentic streptokinase (47 K). Since the cloned streptokinase gene encoded a 47 K mature protein, the lowered molecular weight of S. sanguis streptokinase may reflect posttranslational proteolytic cleavage, which leaves the biological activity of the gene product and its serological reactivity unimpaired.

L11 ANSWER 104 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
 ACCESSION NUMBER: 1984-03868 BIOTECHDS  
 TITLE: Streptokinase: cloning expression and excretion by E.coli; using Streptococcus equisimilis genomic DNA (conference abstract)

AUTHOR: Malke H; Ferretti J J  
 LOCATION: Acad. Sci. GDR, Jena, DDR.  
 SOURCE: Abstr.Annu.Meet.Am.Soc.Microbiol; (1984) 84 Meet., 67  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB Genomic DNA from Streptococcus equisimilis H46A (group C) was cloned into Escherichia coli using the lambda replacement vector L47 and an in vitro packaging system. The phage bank was screened for recombinants containing the streptokinase (skc) gene by the casein-plasminogen overlay technique. 10 Independent

clones containing the **skc** gene were isolated and one was used to subclone the **skc** gene into E.coli plasmid vectors pBR322 and pACYC184. Plasmid pMF2 and pMF5 were among the recombinant plasmids determining **streptokinase** production in 3 different E.coli host strains. Expression of **skc** was independent of its orientation in either vector, indicating that its own promoter was present and functional in E.coli. Analysis of cell free culture supernatant fluids, osmotic shockates, and sonicates of osmotically shocked cells for **streptokinase** activity revealed the substance to be present in all 3 locations, indicating that E.coli cells were capable of releasing substantial amounts of **streptokinase** into the culture medium. (0 ref)

L11 ANSWER 105 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1984:100214 BIOSIS  
DOCUMENT NUMBER: PREV198427016706; BR27:16706  
TITLE: HYBRIDIZATION OF A CLONED GROUP C STREPTOCOCCAL STREPTO KINASE GENE WITH DNA FROM OTHER STREPTOCOCCAL SPECIES.  
AUTHOR(S): HUANG T-T [Reprint author]; WEEKS C R; MALKE H; FERRETTI J J  
CORPORATE SOURCE: UNIV OKLA HEALTH SCI CENT, OKLAHOMA CITY, OKLA, USA  
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D75.  
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.  
ABSTR ANNU MEET AM SOC MICROBIOL.  
CODEN: ASMACK. ISSN: 0094-8519.  
DOCUMENT TYPE: Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH

L11 ANSWER 106 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1984:92832 BIOSIS  
DOCUMENT NUMBER: PREV198427009324; BR27:9324  
TITLE: STREPTO KINASE CLONING EXPRESSION AND EXCRETION BY ESCHERICHIA-COLI.  
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J  
CORPORATE SOURCE: ACAD SCI GDR, JENA, GDR  
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D97.  
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.  
ABSTR ANNU MEET AM SOC MICROBIOL.  
CODEN: ASMACK. ISSN: 0094-8519.  
DOCUMENT TYPE: Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH

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(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

L1 42118 S STREPTOKINASE?  
L2 630 S "SKC" OR "SKC-2"  
L3 42539 S L1 OR L2  
L4 7454183 S CLON? OR EXPRESS? OR RECOMBINANT  
L5 4154 S L3 AND L4  
L6 1362 S EQUISIMILIS



L7 220 S L5 AND L6  
 L8 49433 S INCLUSION (W) BOD?  
 L9 5 S L7 AND L8  
 L10 1 DUP REM L9 (4 DUPLICATES REMOVED)  
 L11 106 DUP REM L7 (114 DUPLICATES REMOVED)  
 L12 5 S LAMNDA

=> s l11 and inclusion  
 L13 1 L11 AND INCLUSION

=> s l11 and aggregat?  
 L14 0 L11 AND AGGREGAT?

=> s l11 and insoluble  
 L15 0 L11 AND INSOLUBLE

=> s l11 and solubl?  
 L16 1 L11 AND SOLUBL?

=> s l11 and coli  
 L17 71 L11 AND COLI

=> d 1-71 ibib ab

L17 ANSWER 1 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 2002322701 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12065504  
 TITLE: Dual control of **streptokinase** and streptolysin S  
 production by the covRS and fasCAX two-component regulators  
 in *Streptococcus dysgalactiae* subsp. **equisimilis**.  
 AUTHOR: Steiner Kerstin; Malke Horst  
 CORPORATE SOURCE: Institute for Molecular Biology, Friedrich Schiller  
 University Jena, D-07745 Jena, Germany.  
 SOURCE: Infection and immunity, (2002 Jul) 70 (7) 3627-36.  
 Journal code: 0246127. ISSN: 0019-9567.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-AY075106; GENBANK-AY075107  
 ENTRY MONTH: 200207  
 ENTRY DATE: Entered STN: 20020615  
 Last Updated on STN: 20020731  
 Entered Medline: 20020730

AB Synthesis of the plasminogen activator **streptokinase** (SK) by group A streptococci (GAS) has recently been shown to be subject to control by two two-component regulators, covRS (or csrRS) and fasBCA. In independent studies, response regulator CovR proved to act as the repressor, whereas FasA was found to act indirectly as the activator by controlling the **expression** of a stimulatory RNA, fasX. In an attempt at understanding the regulation of SK production in the human group C streptococcal (GCS) strain H46A, the strongest SK producer known yet, we provide here physical and functional evidence for the presence of the cov and fas systems in GCS as well and, using a mutational approach, compare the balance between their opposing actions in H46A and GAS strain NZ131. Sequence analysis combined with Southern hybridization revealed that the covRS and fasCAX operons are preserved at high levels of primary structure identity between the corresponding GAS and GCS genes, with the exception of fasB, encoding a second sensor kinase that is not a member of the GCS fas operon. This analysis also showed that wild-type H46A is actually a derepressed mutant for SK and streptolysin S (SLS) synthesis, carrying a K102 amber mutation in covR. Using cov and fas mutations in various combinations together with strain constructs allowing complementation in trans, we found that, in H46A, cov and fas contribute

to approximately equal negative and positive extents, respectively, to constitutive SK and SLS activity. The amounts of SK paralleled the level of *skc*(H46A) transcription. The most profound difference between H46A and NZ131 regarding the relative activities of the *cov* and *fas* systems consisted in significantly higher activity of a functional *CovR* repressor in NZ131 than in H46A. In NZ131, *CovR* decreased SK activity in a *Fas*(+) background about sevenfold, compared to a 1.9-fold reduction of SK activity in H46A. Combined with the very short-lived nature of *covR* mRNA (decay rate, 1.39/min), such differences may contribute to strain-specific peculiarities of the expression of two prominent streptococcal virulence factors in response to environmental changes.

L17 ANSWER 2 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 2002053807 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 11779212  
 TITLE: Specificity role of the **streptokinase** C-terminal domain in plasminogen activation.  
 AUTHOR: Kim Dong Min; Lee Sang Jun; Yoon Suk Kwon; Byun Si Myung  
 CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 305-701 Taejeon, South Korea.  
 SOURCE: Biochemical and biophysical research communications, (2002 Jan 11) 290 (1) 585-8.  
 Journal code: 0372516. ISSN: 0006-291X.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200202  
 ENTRY DATE: Entered STN: 20020125  
 Last Updated on STN: 20020226  
 Entered Medline: 20020225

AB Several pathogenic bacteria secrete plasminogen activator proteins. **Streptokinase** (SKe) produced by *Streptococcus equisimilis* and staphylokinase secreted from *Staphylococcus aureus* are human plasminogen activators and **streptokinase** (SKu), produced by *Streptococcus uberis*, is a bovine plasminogen activator. Thus, the fusion proteins among these activators can explain the function of each domain of SKe. Replacement of the SKalpha domain with staphylokinase donated the staphylokinase-like activation activity to SKe, and the SKbetagamma domain played a role of nonproteolytic activation of plasminogen. Recombinant SKu also activated human plasminogen by staphylokinase-like activation mode. Because SKu has homology with SKe, the bovine plasminogen activation activities of SKe fragments were checked. SKbetagamma among them had activation activity with bovine plasminogen. This means that the C-terminal domain (gamma-domain) of **streptokinase** determines plasminogen species necessary for activation and converses the ability of substrate recognition to human species.  
 (c)2002 Elsevier Science.

L17 ANSWER 3 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 2000038313 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 10569766  
 TITLE: Cloning, **expression**, sequence analysis, and characterization of **streptokinases** secreted by porcine and equine isolates of *Streptococcus equisimilis*.  
 AUTHOR: Caballero A R; Lottenberg R; Johnston K H  
 CORPORATE SOURCE: Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans, Louisiana 70112, USA.  
 CONTRACT NUMBER: R01DK45014 (NIDDK)

SOURCE: Infection and immunity, (1999 Dec) 67 (12) 6478-86.  
Journal code: 0246127. ISSN: 0019-9567.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF104300; GENBANK-AF104301  
ENTRY MONTH: 199912  
ENTRY DATE: Entered STN: 20000113  
Last Updated on STN: 20000113  
Entered Medline: 19991220

AB **Streptokinases** secreted by nonhuman isolates of group C streptococci (*Streptococcus equi*, *S. equisimilis*, and *S. zooepidemicus*) have been shown to bind to different mammalian plasminogens but exhibit preferential plasminogen activity. The **streptokinase** genes from *S. equisimilis* strains which activated either equine or porcine plasminogen were **cloned**, sequenced, and **expressed** in *Escherichia coli*. The **streptokinase** secreted by the equine isolate had little similarity to any known **streptokinases** secreted by either human or porcine isolates. The **streptokinase** secreted by the porcine isolate had limited structural and functional similarities to **streptokinases** secreted by human isolates. Plasminogen activation studies with immobilized (His)(6)-tagged **recombinant streptokinases** indicated that these **recombinant streptokinases** interacted with plasminogen in a manner similar to that observed when **streptokinase** and plasminogen interact in the fluid phase. Analysis of the cleavage products of the **streptokinase**-plasminogen interaction indicated that human, equine, and porcine plasminogens were all cleaved at the same highly conserved site. The site at which **streptokinase** was cleaved to form altered **streptokinase** (Sk\*) was also determined. This study confirmed not only the presence of **streptokinases** in nonhuman *S. equisimilis* isolates but also that these proteins belong to a family of plasminogen activators more diverse than previously thought.

L17 ANSWER 4 OF 71 MEDLINE on STN

ACCESSION NUMBER: 1998350778 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9686161

TITLE: **Cloning, expression and purification of recombinant streptokinase: partial characterization of the protein expressed in Escherichia coli.**

AUTHOR: Avilan L; Yarzabal A; Jurgensen C; Bastidas M; Cruz J; Puig J

CORPORATE SOURCE: Laboratorio de Biología y Medicina Experimental, Facultad de Ciencias, Universidad de Los Andes, Mérida, Venezuela.

SOURCE: Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.], (1997 Dec) 30 (12) 1427-30.

Journal code: 8112917. ISSN: 0100-879X.

PUB. COUNTRY: Brazil

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199810

ENTRY DATE: Entered STN: 19981020

Last Updated on STN: 19981020

Entered Medline: 19981005

AB We cloned the **streptokinase** (STK) gene of *Streptococcus equisimilis* in an **expression** vector of *Escherichia coli* to overexpress the profibrinolytic protein under the control of a tac promoter. Almost all the **recombinant**

STK was exported to the periplasmic space and recovered after gentle lysozyme digestion of induced cells. The periplasmic fraction was chromatographed on DEAE Sepharose followed by chromatography on phenyl-agarose. Active proteins eluted between 4.5 and 0% ammonium sulfate, when a linear gradient was applied. Three major STK derivatives of 47.5 kDa, 45 kDa and 32 kDa were detected by Western blot analysis with a polyclonal antibody. The 32-kDa protein formed a complex with human plasminogen but did not exhibit Glu-plasminogen activator activity, as revealed by a zymographic assay, whereas the 45-kDa protein showed a  $K(m) = 0.70 \text{ microM}$  and  $k_{cat} = 0.82 \text{ s}^{-1}$ , when assayed with a chromogen-coupled substrate. These results suggest that these proteins are putative fragments of STK, possibly derived from partial degradation during the export pathway or the purification steps. The 47.5-kDa band corresponded to the native STK, as revealed by peptide sequencing.

L17 ANSWER 5 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 96397500 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8804394  
 TITLE: Cloning of heterologous genes specifying detrimental proteins on pUC-derived plasmids in *Escherichia coli*.  
 AUTHOR: Muller J; van Dijl J M; Venema G; Bron S  
 CORPORATE SOURCE: Institut fur Molekularbiologie, Friedrich-Schiller-Universitat Jena, Germany.  
 SOURCE: Molecular & general genetics : MGG, (1996 Aug 27) 252 (1-2) 207-11.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199610  
 ENTRY DATE: Entered STN: 19961219  
 Last Updated on STN: 19961219  
 Entered Medline: 19961031

AB A system is described that enables the cloning of genes specifying detrimental proteins in *Escherichia coli*. The system is based on pUC plasmids and was developed for the **expression** of the *Bacillus subtilis* *csaA* gene, which is lethal when **expressed** at high levels. Suppressor strains that tolerate the presence of plasmids for high-level **expression** of *csaA* were isolated, which contained small cryptic deletion variants of the parental plasmid in high copy numbers. The cryptic plasmids consisted mainly of the pUC replication functions and lacked the *csaA* region and selectable markers. The co-resident, incompatible, cryptic plasmids enabled the maintenance of the *csaA* plasmids by reducing their copy number 20-fold, which resulted in a concomitant 3- to 7-fold reduction in the **expression** of plasmid-encoded genes. Strains carrying these cryptic endogenous plasmids proved to be useful for the construction of pUC-based **recombinant** plasmids carrying other genes, such as the *skc* gene of *Streptococcus equisimilis*, which cannot be cloned in high copy numbers in *E. coli*. Several strategies to reduce production levels of heterologous proteins specified by plasmids are compared.

L17 ANSWER 6 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 96396845 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8803948  
 TITLE: Structural dissection and functional analysis of the complex promoter of the **streptokinase** gene from *Streptococcus equisimilis* H46A.  
 AUTHOR: Grafe S; Ellinger T; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Medical microbiology and immunology, (1996 May) 185 (1)

11-7.

Journal code: 0314524. ISSN: 0300-8584.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199701

ENTRY DATE: Entered STN: 19970219

Last Updated on STN: 19970219

Entered Medline: 19970131

AB The overlapping tandem promoters of the *streptokinase* gene, P1 and P2, identified previously by S1 nuclease transcript mapping were functionally dissected by mutagenesis of their -10 regions and fused transcriptionally with or without the 202-bp upstream region (USR) to the luciferase reporter gene (luc) from *Photinus pyralis* to analyze the contribution of the different sequence elements to promoter activity in *Escherichia coli* and the homologous *Streptococcus equisimilis* strain H46A. In *E. coli*, virtually the entire promoter activity derived from the upstream promoter P1. In *S. equisimilis*, luc expression increased in the following order of the involved sequence elements: P2 approximately equal to P2 + USR < P1 < P1 + P2 < P1 + USR < P1 + P2 + USR. This shows that (1) in the homologous system, P1 and P2 alone are extremely weak, (2) in the USR-less arrangement, only the combined core promoters have substantial activity, and (3) the USR stimulates only P1 and the combination of P1 + P2. Thus, the tandem promoters presumably function by mutual contributory action and their full activity strongly depends on the AT-rich and statically bent upstream region. The distinctive feature determining the strength of P1 in both hosts appears to be its extended -10 region which matches the consensus TRTGN established for strong *S. pneumoniae* and *Bacillus subtilis* promoters.

L17 ANSWER 7 OF 71 MEDLINE on STN

ACCESSION NUMBER: 96305364 MEDLINE

DOCUMENT NUMBER: PubMed ID: 8706717

TITLE: Cloning, sequencing and functional overexpression of the *Streptococcus equisimilis* H46A gapC gene encoding a glyceraldehyde-3-phosphate dehydrogenase that also functions as a plasmin(ogen)-binding protein. Purification and biochemical characterization of the protein.

AUTHOR: Gase K; Gase A; Schirmer H; Malke H

CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.

SOURCE: European journal of biochemistry / FEBS, (1996 Jul 1) 239 (1) 42-51.

Journal code: 0107600. ISSN: 0014-2956.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-X97788

ENTRY MONTH: 199609

ENTRY DATE: Entered STN: 19960919

Last Updated on STN: 19990129

Entered Medline: 19960910

AB We previously identified DNA sequences involved in the function of the complex promoter of the *streptokinase* gene from *Streptococcus equisimilis* H46A, a human serogroup C strain known to express this gene at a high level. As a prerequisite to understanding possible mechanisms that control the balance between the plasminogen activating and plasmin(ogen) binding capacities of H46A, we describe here its gapC gene encoding glyceraldehyde-3-phosphate dehydrogenase (Gap-DH, EC 1.2.1.12), a glycolytic enzyme apparently transported to the cell surface where it functions as a

plasmin(ogen).binding protein. The gapC gene was cloned and sequenced and found to code for a 336-amino-acid polypeptide (approximately 35.9 kDa) exhibiting 94.9% sequence identity to the Plr protein from *Streptococcus pyogenes* shown by others to be capable of plasmin binding [Lottenberg, R., Broder, C. C., Boyle, M. D., Kain, S. J., Schroeder, B. L. & Curtiss, R. III (1992) *J. Bacteriol.* 174, 5204-5210]. To study the properties of the GapC protein, its gene was inducibly overexpressed in *Escherichia coli* from QIAexpress expression plasmids to yield the authentic GapC or (His)6GapC carrying a hexahistidyl N-terminus to permit affinity purification. Both proteins were functionally active, exhibiting specific GraP-DH activities of about 80 kat/mol (approximately 130 U/mg) after purification. Their binding parameters [association (ka) and dissociation (kd) rate constants, and equilibrium dissociation constants (Kd = kd/ka)] for the interaction with human Gluplasminogen and plasmin were determined by real-time biospecific interaction analysis using the Pharmacia BIAcore instrument. For comparative purposes, the commercial GraP-DH from *Bacillus stearothermophilus* (BstGraP-DH), a nonpathogenic organism, was included in these experiments. The Kd values for binding of plasminogen to GapC, (His)6GapC and BstGraP-DH were 220 nM, 260 nM and 520 nM, respectively, as compared to 25 nM, 17 nM and 98 nM, respectively, for the binding to plasmin. These data show that both the zymogen and active enzyme possess low-affinity binding sites for the gapC gene product and that the hexahistidyl terminus does not affect its function. Prior limited treatment with plasmin enhanced the subsequent plasminogen binding capacity of all three GraP-DHs, presumably by the exposure of new C-terminal lysine residues for binding to the zymogen.

L17 ANSWER 8 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 96200111 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8631718  
 TITLE: Functional analysis of a relA/spoT gene homolog from *Streptococcus equisimilis*.  
 AUTHOR: Mechold U; Cashel M; Steiner K; Gentry D; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Journal of bacteriology, (1996 Mar) 178 (5) 1401-11.  
 Journal code: 2985120R. ISSN: 0021-9193.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199607  
 ENTRY DATE: Entered STN: 19960715  
 Last Updated on STN: 19970203  
 Entered Medline: 19960703

AB We examined the functional attributes of a gene encountered by sequencing the streptokinase gene region of *Streptococcus equisimilis* H46A. This gene, originally called rel, here termed relS. *equisimilis*, is homologous to two related *Escherichia coli* genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of *E. coli* mutants led us to deduce that the highly expressed rel S. *equisimilis* gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel S. *equisimilis* protein, purified from an *E. coli* relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel S. *equisimilis* protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel S. *equisimilis* protein displays immunological

cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel S. *equisimilis* gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel S. *equisimilis* in S. *equisimilis* abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L17 ANSWER 9 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 96001243 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 7557478  
 TITLE: Secretion of **streptokinase** fusion proteins from Escherichia coli cells through the hemolysin transporter.  
 AUTHOR: Kern I; Ceglowski P  
 CORPORATE SOURCE: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa.  
 SOURCE: Gene, (1995 Sep 22) 163 (1) 53-7.  
 Journal code: 7706761. ISSN: 0378-1119.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199511  
 ENTRY DATE: Entered STN: 19951227  
 Last Updated on STN: 20021008  
 Entered Medline: 19951108

AB The hemolysin (HlyA) secretion system was used to achieve the sec-independent secretion of **streptokinase** (Skc) originating from Streptococcus *equisimilis* into the medium by Escherichia coli cells. The in-frame fusions of the **skc** gene, either possessing or lacking a region encoding the signal peptide (SP) with the 3'-end of the hlyA gene of various lengths were analysed. All hybrids retained Skc activity. Hybrid proteins devoided of the N-terminal SP, regardless of length of the hlyA secretion signal (62 vs. 194 amino acids), were secreted into the medium by the E. coli HlyA transporter at similar levels. Considerable amounts of hybrid proteins were still, however, associated with E. coli cells, mainly in the degraded form.

L17 ANSWER 10 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 95342169 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 7616967  
 TITLE: Complex transcriptional control of the **streptokinase** gene of Streptococcus *equisimilis* H46A.  
 AUTHOR: Gase K; Ellinger T; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Molecular & general genetics : MGG, (1995 Jun 25) 247 (6) 749-58.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199508  
 ENTRY DATE: Entered STN: 19950905  
 Last Updated on STN: 19950905  
 Entered Medline: 19950822

AB On the Streptococcus *equisimilis* H46A chromosome, the divergent coding sequences of the genes for the plasminogen activator **streptokinase** (skc) and a leucine-rich protein (lrp), the function of which is unknown, are separated by a 328 bp intrinsically bent DNA region rich in AT tracts. To begin to understand the **expression** control of these two genes, we mapped their

transcriptional initiation sites by S1 nuclease analysis and studied the influence of the bent intergenic region on promoter strength, using promoter-reporter gene fusions of *skc*' and *lrp*' to 'lacZ from *Escherichia coli*. The major transcriptional start sites, in both *S. equisimilis* and *E. coli*, mapped 22 bases upstream of the ATG start site of *lrp* (G), and 24 and 32 bases upstream of the translational initiation codon of *skc* (A and G, respectively), indicating the existence of two overlapping canonical *skc* promoters arranged in tandem on opposite faces of the helix. The reporter gene fusions were cloned in *E. coli* on a vector containing a 1.1 kb fragment of the *S. equisimilis* *dexB* gene, thus allowing promoter strength to be measured in multiple plasmid-form copies in the heterologous host and in single-copy genomic form following integration into the *skc* region of the homologous host. In *S. equisimilis*, *skc*'-'lacZ was expressed about 200-fold more strongly than the corresponding *lrp*'-'lacZ fusion. In contrast, in *E. coli*, the corresponding levels of expression differed by only about 11-fold. Deletion of the 202 bp bent region upstream of the *skc* and *lrp* core promoters caused a 13-fold decrease in *skc* promoter activity in *S. equisimilis* but did not alter *lrp* promoter strength in this host. In contrast, when studied in *E. coli*, this deletion did not alter the strength of the *skc*-double promoter and even increased by 2.4- to 3-fold the activity of the *lrp* promoter. This comparative promoter analysis shows that *skc* has a complex promoter structure, the activity of which in the homologous genomic environment specifically depends on sequences upstream of the two core promoters. Thus, the *skc* promoter structure resembles that of an array of promoters involved in a transcriptional switch; however, the nature of the potential switch factor(s) remains unknown.

L17 ANSWER 11 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 95157528 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 7531815  
 TITLE: Transcription termination of the *streptokinase* gene of *Streptococcus equisimilis* H46A: bidirectionality and efficiency in homologous and heterologous hosts.  
 AUTHOR: Steiner K; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Molecular & general genetics : MGG, (1995 Feb 6) 246 (3) 374-80.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199503  
 ENTRY DATE: Entered STN: 19950322  
 Last Updated on STN: 19960129  
 Entered Medline: 19950316

AB In *Streptococcus equisimilis* H46A, a hypersymmetrical transcription terminator with bidirectional activity was localized between the translational termination codons of the *streptokinase* gene, *skc*, and the *rel-orf1* genes. These two transcription units are oriented towards each other, and under normal conditions the *skc* mRNA level exceeds that of the *rel-orf1* genes by a factor of at least 1000. Reporter vectors based on the promoterless *cat* gene were constructed by transcriptional fusion of *skc* to *cat*, such that the region between the two genes contained the terminator in *skc* orientation or in *rel-orf1* orientation. Additionally, *skc* and *cat* were fused directly, with deletion of the terminator. The reporter vectors were designed to be capable of being studied either as multicopy plasmids in *Escherichia coli* or in single copy following



integration, via *skc*, into the *S. equisimilis* chromosome. Chloramphenicol acetyl transferase (CAT) activity assays in conjunction with determination of chloramphenicol resistance levels and Northern hybridization analysis showed that the terminator is active in either host and orientation. However, termination efficiency was host dependent, with high terminator strength being observed in the homologous streptococcal background and appreciable readthrough occurring in *E. coli*. The extent of transcriptional readthrough was dependent upon terminator orientation, with termination being more efficient in *rel-orf1* polarity. The results suggest that, in *S. equisimilis*, transcription of both *skc* and *rel-orf1* is efficiently terminated by a common signal, and that these genes are largely protected from convergent transcription, which otherwise would seem to be particularly detrimental to the weakly expressed *rel-orf1* genes.

L17 ANSWER 12 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 94049672 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8232196  
 TITLE: Genetic organization of the *streptokinase* region of the *Streptococcus equisimilis* H46A chromosome.  
 AUTHOR: Mechold U; Steiner K; Vettermann S; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Molecular & general genetics : MGG, (1993 Oct) 241 (1-2) 129-40.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-X72832  
 ENTRY MONTH: 199312  
 ENTRY DATE: Entered STN: 19940117  
 Last Updated on STN: 19940117  
 Entered Medline: 19931215

AB The complete nucleotide sequences of four genes and one open reading frame (ORF1) adjacent to the *streptokinase* gene, *skc*, from *Streptococcus equisimilis* H46A were determined. These genes are encoded on the opposite DNA strand to *skc* and are arranged as follows: *dexB-abc-lrp-skc-ORF1-rel*. The *dexB* gene, coding for an alpha-glucosidase (M(r) 61,733), and *abc*, encoding an ABC transporter (M(r) 42,080), are similar to the *dexB* and *msmK* genes, respectively, from the multiple sugar metabolism operon of *S. mutans*. The *lrp* gene specifies a leucine-rich protein (M(r) 32,302) that has a leucine-zipper motif at its C-terminus. The function of the Lrp protein is not known but appeared to be detrimental when overexpressed in *Escherichia coli*. Although *lrp* appears not to be an essential gene, as judged by plasmid insertion mutagenesis, it is conserved in all streptococcal strains carrying a *streptokinase* gene. The *rel* gene showed significant homology to the *E. coli* *relA* and *spoT* genes involved in the stringent response to amino acid deprivation. Multiple alignment of the amino acid sequences of Rel (M(r) 83,913), RelA and SpoT revealed 59.4% homology of the primary structures. Northern hybridization analyses of the genes in the *skc* region showed *skc* to be transcribed most abundantly. In addition to transcripts for *skc*, monocistronic mRNAs were detected for all three genes divergently transcribed from *skc*. Although there was also some read-through transcription from *lrp* into *abc*, and from *abc* into *dexB*, the transcription pattern suggests a high degree of transcriptional and functional independence not only of *skc* but also *abc* and *dexB*. Prominent structural features in intergenic regions included a static DNA bending locus located upstream and a putative bidirectional transcription terminator downstream of *skc*.

L17 ANSWER 13 OF 71 MEDLINE on STN

ACCESSION NUMBER: 92039051 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 1937032  
 TITLE: Isolation, sequence and expression in *Escherichia coli*, *Bacillus subtilis* and *Lactococcus lactis* of the DNase (streptodornase)-encoding gene from *Streptococcus equisimilis* H46A.  
 AUTHOR: Wolinowska R; Ceglowski P; Kok J; Venema G  
 CORPORATE SOURCE: Department of Pharmaceutical Microbiology, Medical Academy, Warsaw, Poland.  
 SOURCE: Gene, (1991 Sep 30) 106 (1) 115-9.  
 Journal code: 7706761. ISSN: 0378-1119.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-M59725; GENBANK-M59726; GENBANK-M59727;  
 GENBANK-M59728; GENBANK-M63990; GENBANK-S61507;  
 GENBANK-S63856; GENBANK-S63863; GENBANK-S65020;  
 GENBANK-S65060; GENBANK-X17241  
 ENTRY MONTH: 199112  
 ENTRY DATE: Entered STN: 19920124  
 Last Updated on STN: 19920124  
 Entered Medline: 19911223

AB A partial library of BclI-generated chromosomal DNA fragments from *Streptococcus equisimilis* H64A (Lancefield Group C) was constructed in *Escherichia coli*. Clones displaying either streptokinase or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdC) expressing the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdC gene was expressed in *E. coli*, *Bacillus subtilis* and *Lactococcus lactis*. As observed for *S. equisimilis*, in the heterologous Gram + hosts, at least part of the SDC protein was secreted into the medium.

L17 ANSWER 14 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 90172183 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 2625666  
 TITLE: Sequence-directed DNA bending upstream of the streptokinase promoter.  
 AUTHOR: Muller J; Malke H  
 CORPORATE SOURCE: Akademie der Wissenschaften der DDR.  
 SOURCE: Journal of basic microbiology, (1989) 29 (9) 611-6.  
 Journal code: 8503885. ISSN: 0233-111X.  
 PUB. COUNTRY: GERMANY, EAST: German Democratic Republic  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199004  
 ENTRY DATE: Entered STN: 19900601  
 Last Updated on STN: 19900601  
 Entered Medline: 19900409

AB A 450-base pair (bp) HinfI restriction fragment from the chromosome of *Streptococcus equisimilis* H46A contains the early coding region of the streptokinase gene (skc), the skc promoter, and a stretch of DNA 5' to the --35 region of the skc promoter. Two-dimensional polyacrylamide (PA) gel electrophoresis at two different temperatures showed that this fragment migrates anomalously slowly on PA gels, suggesting the existence of a bent DNA conformation. Inspection of the nucleotide sequence confirmed this suggestion by revealing numerous oligomeric dA.dT tracts, some of which are in phase with the helix screw. Computer analysis of the sequence predicted the

existence of two bending loci, one of which is located upstream of the **skc** promoter. In addition to showing DNA bending, the 450-bp **HinfI** fragment contains multiple 13-bp sequences homologous to the *Escherichia coli* integration host factor DNA-binding consensus sequence. Insertion of **IS1** into a site immediately upstream of the--35 region decreased the **expression** level of **skc** in *E. coli*, suggesting that DNA conformation upstream of the promoter has a role in **skc expression**.

L17 ANSWER 15 OF 71 MEDLINE on STN

ACCESSION NUMBER: 88302119 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 3043172  
TITLE: Tripartite **streptokinase** gene fusion vectors for gram-positive and gram-negative procaryotes.  
AUTHOR: Klessen C; Schmidt K H; Ferretti J J; Malke H  
CORPORATE SOURCE: Academy of Sciences of the GDR, Central Institute of Microbiology and Experimental Therapy, Jena.  
SOURCE: Molecular & general genetics : MGG, (1988 May) 212 (2) 295-300.  
Journal code: 0125036. ISSN: 0026-8925.  
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198809  
ENTRY DATE: Entered STN: 19900308  
Last Updated on STN: 19900308  
Entered Medline: 19880921

AB A specific 1,596 bp **HincII** fragment ('**skc**') from the chromosome of *Streptococcus equisimilis* contains an active **streptokinase** (SK) gene (**skc**) lacking, in addition to the **expression** signals, codons 1 through 39 of wild-type **skc** but retaining the remainder of the **skc** coding sequence together with the transcription terminator. Using this fragment as an indicator gene, we constructed two types of vectors which in appropriate hosts resulted in the synthesis of SK fusion proteins after insertional activation of '**skc**'. The first type are open reading frame (ORF) vectors in which '**skc**' was inserted into pUC18 out of frame with respect to **lacZ**', thus conferring an SK-negative phenotype. Any DNA fragments representing ORFs inserted between the **lacZ**' **expression** signals and '**skc**' such that the **skc** reading frame was restored resulted in the production of tripartite proteins which exhibited SK activity. The second type of vector, which functioned in both gram-positive and gram-negative bacteria, used the streptococcal **speA expression** and secretion signals in front of the ORF to activate '**skc**' insertionaly. Using a large fragment from the chymosin gene as the target sequence, the usefulness of these vectors for studying foreign gene **expression** in streptococci as well as *Escherichia coli* was demonstrated.

L17 ANSWER 16 OF 71 MEDLINE on STN

ACCESSION NUMBER: 84221999 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 6374659  
TITLE: **Streptokinase: cloning, expression, and excretion by Escherichia coli.**  
AUTHOR: Malke H; Ferretti J J  
CONTRACT NUMBER: AI 9304 (NIAID)  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1984 Jun) 81 (11) 3557-61.  
Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English

FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198407  
ENTRY DATE: Entered STN: 19900320  
Last Updated on STN: 19970203  
Entered Medline: 19840724

AB Genomic DNA from *Streptococcus equisimilis* strain H46A was cloned in *Escherichia coli* by using the bacteriophage lambda replacement vector L47 and an in vitro packaging system. A casein/plasminogen overlay technique was used to screen the phage bank for recombinants carrying the streptokinase gene (skc). The gene was present with a frequency of 1 in 836 recombinants, and 10 independent clones containing skc were isolated and physically characterized. One recombinant clone was used to subclone skc in *E. coli* plasmid vectors. Plasmid pMF2 [10.4 kilobases (kb)] consisting of pACYC184 with a 6.4-kb H46A DNA fragment in the EcoRI site and pMF5 (6.9 kb) carrying a 2.5-kb fragment in the Pst I site of pBR322 were among the recombinant plasmids determining streptokinase production in three different *E. coli* host strains. Expression of skc was independent of its orientation in either vector, indicating that its own promoter was present and functional in *E. coli*. However, expression in pBR322 was more efficient in one orientation than in the other, suggesting that one or both of the bla gene promoters contributed to skc expression. Several lines of evidence, including proof obtained by the immunodiffusion technique, established the identity of *E. coli* streptokinase. Testing cell-free culture supernatant fluids, osmotic shock fluids, and sonicates of osmotically shocked cells for streptokinase activity revealed the substance to be present in all three principal locations, indicating that *E. coli* cells were capable of releasing substantial amounts of streptokinase into the culture medium.

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ACCESSION NUMBER: 2005441717 EMBASE  
TITLE: Heberkinasa: Recombinant streptokinase [9].  
AUTHOR: Hernandez L.; Martinez Y.; Quintana M.; Besada V.; Martinez E.  
CORPORATE SOURCE: L. Hernandez, Production Division, Centro de Ingenieria Genetica Y Biotecnologia, Ave 31 e/ 158 y 190, Cubanacan, Playa, Habana 0600, Cuba. luciano.hernandez@cigb.edu.cu  
SOURCE: European Heart Journal, (2005) Vol. 26, No. 16, pp. 1691.  
Refs: 4  
ISSN: 0195-668X CODEN: EHJODF  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Letter  
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery  
030 Pharmacology  
037 Drug Literature Index  
LANGUAGE: English  
ENTRY DATE: Entered STN: 20051020  
Last Updated on STN: 20051020

L17 ANSWER 18 OF 71 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2002110457 EMBASE  
TITLE: Can imperfections help to improve bioreactor performance?.  
AUTHOR: Patnaik P.R.  
CORPORATE SOURCE: P.R. Patnaik, Institute of Microbial Technology, Sector 39-A, Chandigarh-160 036, India. pratap@imtech.res.in  
SOURCE: Trends in Biotechnology, (1 Apr 2002) Vol. 20, No. 4, pp. 135-137.

Refs: 22  
ISSN: 0167-7799 CODEN: TRBIDM  
PUBLISHER IDENT.: S 0167-7799(01)01922-9  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; General Review  
FILE SEGMENT: 004 Microbiology  
027 Biophysics, Bioengineering and Medical  
Instrumentation

LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 20020404  
Last Updated on STN: 20020404

AB Pilot-scale and larger bioreactors differ from small laboratory-scale reactors in terms of a greater occurrence of noise and incomplete mixing of the broth. Conventional control tries to induce good mixing and to filter out the noise as completely as possible. As such an 'ideal' operation is difficult to achieve, recent work has tried to exploit the non-ideal features to improve the performance. Using artificial neural networks, the degree of mixing, the extent of filtering of noise and the distribution of plasmid copy number (in a **recombinant** fermentation) can be controlled effectively on-line. This strategy generates better productivities than well-mixed noise-free operations, which suggests that deviations from ideal behaviour should be gainfully harnessed and not suppressed.

L17 ANSWER 19 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:96119 BIOSIS

DOCUMENT NUMBER: PREV200000096119

TITLE: Two **streptokinase** genes are **expressed** with different solubility in *Escherichia coli* W3110.

AUTHOR(S): Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis

CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba

SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.

CODEN: BILED3. ISSN: 0141-5492.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 15 Mar 2000

Last Updated on STN: 3 Jan 2002

AB The **streptokinase** (SK) gene from *S. equisimilis* H46A (ATCC 12449) was cloned in *E. coli* W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from *S. equisimilis* (ATCC 9542) which had been cloned in *E. coli* W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of inclusion bodies.

L17 ANSWER 20 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:88244 BIOSIS

DOCUMENT NUMBER: PREV200000088244

TITLE: Hydrodynamic radii of native and denatured proteins measured by pulse field gradient NMR techniques.

AUTHOR(S): Wilkins, Deborah K.; Grimshaw, Shaun B.; Receveur, Veronique; Dobson, Christopher M.; Jones, Jonathan A.; Smith, Lorna J. [Reprint author]

CORPORATE SOURCE: Oxford Centre for Molecular Sciences, New Chemistry

Laboratory, University of Oxford, South Parks Road, Oxford,  
OX1 3QT, UK  
SOURCE: Biochemistry, (Dec. 14, 1999) Vol. 38, No. 50, pp.  
16424-16431. print.  
CODEN: BICHAW. ISSN: 0006-2960.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 10 Mar 2000  
Last Updated on STN: 3 Jan 2002

AB Pulse field gradient NMR methods have been used to determine the effective hydrodynamic radii of a range of native and nonnative protein conformations. From these experimental data, empirical relationships between the measured hydrodynamic radius ( $R_h$ ) and the number of residues in the polypeptide chain ( $N$ ) have been established; for native folded proteins  $R_h = 4.75N^{0.29}$  Å and for highly denatured states  $R_h = 2.21N^{0.57}$  Å. Predictions from these equations agree well with experimental data from dynamic light scattering and small-angle X-ray or neutron scattering studies reported in the literature for proteins ranging in size from 58 to 760 amino acid residues. The predicted values of the hydrodynamic radii provide a framework that can be used to analyze the conformational properties of a range of nonnative states of proteins. Several examples are given here to illustrate this approach including data for partially structured molten globule states and for proteins that are unfolded but biologically active under physiological conditions. These reveal evidence for significant coupling between local and global features of the conformational ensembles adopted in such states. In particular, the effective dimensions of the polypeptide chain are found to depend significantly on the level of persistence of regions of secondary structure or features such as hydrophobic clusters within a conformational ensemble.

L17 ANSWER 21 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1998:317596 BIOSIS  
DOCUMENT NUMBER: PREV199800317596  
TITLE: Effect of signal peptide changes on the extracellular processing of streptokinase from *Escherichia coli*: Requirement for secondary structure at the cleavage junction.  
AUTHOR(S): Pratap, J.; Dikshit, K. L. [Reprint author]  
CORPORATE SOURCE: Inst. Microbial Technology, Sector 39-A, Chandigarh 160 036, India  
SOURCE: Molecular and General Genetics, (May, 1998) Vol. 258, No. 4, pp. 326-333. print.  
CODEN: MGGEAE. ISSN: 0026-8925.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 22 Jul 1998  
Last Updated on STN: 10 Sep 1998

AB Streptokinase (SK), an extracellular protein from *Streptococcus equisimilis*, is secreted post-translationally by *Escherichia coli* using both its native and *E. coli*-derived transport signals. In this communication we report that cleavage specificity of signal peptidase I, and thus efficiency of secretion, varies in *E. coli* when SK export is directed by different transport signals. The native (+ 1) N-terminus of mature SK was retained when it was transported under the control of its own, PelB or LamB signal peptide. However, when translocation of SK was controlled by the OmpA or MalE signal peptide, Ala2 of mature SK was preferred as a cleavage site for the pre-SK processing. Our results indicate that compatibility of the leader peptide with the mature sequences of SK, which fulfils the requirement for a given secondary structure within the cleavage region, is essential for maintaining the correct processing of pre-SK. An OmpA-SK fusion, which results in the deletion of two N-terminal amino acid residues of mature

SK, was further studied with respect to the recognition of alternative cleavage site in *E. coli*. The alanine at +2 in mature SK was changed to glycine or its relative position was changed to +3 by introducing a methionine residue at the +1 position. Both alterations resulted in the correct cleavage of pre-SK at the original OmpA fusion site. In contrast, introduction of an additional alanine at +4, creating three probable cleavage sites (Ala-x-Ala-x-Ala-x-Ala), resulted in the recognition of all three target sites for cleavage, with varying efficiency. The results indicate that the nature of the secondary structure generated at the cleavage junction of pre-SK, resulting from the fusion of different signal peptides, modulates the cleavage specificity of signal peptidase I during extracellular processing of SK. Based on these findings it is proposed that flexibility in the interaction of the active site of signal peptidase I with the cleavage sites of signal peptides may occur when it encounters two or more juxtaposed cleavage sites. Preference for one cleavage site over another, then, may depend on fulfillment of secondary structure requirements in the vicinity of the pre-protein cleavage junction.

L17 ANSWER 22 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1995:547506 BIOSIS  
DOCUMENT NUMBER: PREV199698561806  
TITLE: High-level **expression** and secretion of **streptokinase** in *Escherichia coli*.  
AUTHOR(S): Ko, Jae Hyeong; Park, Do Deun; Kim, Il Chul; Lee, Si Hyoun; Byun, Si Myung [Reprint author]  
CORPORATE SOURCE: Dep. BioSci., Korea Advanced Inst. Sci. Technol., 373-1, Kusung-dong, Yuseong-ku, Taejeon 305-701, South Korea  
SOURCE: Biotechnology Letters, (1995) Vol. 17, No. 10, pp. 1019-1024.  
CODEN: BILED3. ISSN: 0141-5492.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 31 Dec 1995  
Last Updated on STN: 31 Dec 1995

AB The high-level **expression** plasmid for **streptokinase**, pSK100, has been constructed. It contains a tac promoter, an ompA signal sequence, a **streptokinase** structural gene(**skc**) and a rrnBT1T2 transcription terminator. *E. coli* JM 109 carrying pSK100 produced about 5,000IU of **streptokinase** per 1 ml of LB-ampicillin media. About 95% of the **expressed streptokinase** was secreted into the periplasmic and extracellular fractions. The recombinant **streptokinase** in high yield and purity may be a potential alternative source for the therapeutic agent.

L17 ANSWER 23 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1992:340953 BIOSIS  
DOCUMENT NUMBER: PREV199243030503; BR43:30503  
TITLE: **STREPTOKINASE** MUTATIONS AFFECTING **SKC** **EXPRESSION** IN HOMOLOGOUS AND HETEROLOGOUS HOSTS.  
AUTHOR(S): MECHOLD U [Reprint author]; MULLER J; MALKE H  
CORPORATE SOURCE: CENTRAL INST MICROBIOL EXP THERAPY, JENA D-6900, GER  
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1992) pp. 336-338. OREFICI, G. (ED.). ZENTRALBLATT FUER BAKTERIOLOGIE SUPPLEMENT, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS; (INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS); XI LANCEFIELD INTERNATIONAL SYMPOSIUM ON STREPTOCOCCI AND STREPTOCOCCAL DISEASES, SIENA, ITALY, SEPTEMBER 10-14, 1990. XIX+569P. GUSTAV FISCHER VERLAG: STUTTGART, GERMANY; NEW YORK, NEW YORK,

USA. ILLUS.  
Publisher: Series: Zentralblatt fuer Bakteriologie  
Supplement.  
ISSN: 0941-018X. ISBN: 3-437-11362-3, 1-56081-333-4.  
DOCUMENT TYPE: Book  
Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 16 Jul 1992  
Last Updated on STN: 16 Jul 1992

L17 ANSWER 24 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1991:513401 BIOSIS  
DOCUMENT NUMBER: PREV199141114116; BR41:114116  
TITLE: **EXPRESSION AND PROPERTIES OF HYBRID  
STREPTOKINASES EXTENDED BY AMINO-TERMINAL  
PLASMINOGEN KRINGLE DOMAINS.**  
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J  
CORPORATE SOURCE: DEP MICROBIOL IMMUNOL, UNIV OKLA HEALTH SCI CENTER,  
OKLAHOMA CITY, OKLA 73190, USA  
SOURCE: (1991) pp. 184-189. DUNNY, G. M., P. P. CLEARY AND L. L.  
MCKAY (ED.). GENETICS AND MOLECULAR BIOLOGY OF  
STREPTOCOCCI, LACTOCOCCI, AND ENTEROCOCCI; THIRD  
INTERNATIONAL ASM (AMERICAN SOCIETY FOR MICROBIOLOGY)  
CONFERENCE, MINNEAPOLIS, MINNESOTA, USA, JUNE 6-9, 1990.  
VIII+310P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON,  
D.C., USA. ILLUS.  
ISBN: 1-55581-034-9.

DOCUMENT TYPE: Book  
Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 14 Nov 1991  
Last Updated on STN: 14 Nov 1991

L17 ANSWER 25 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1987:499919 BIOSIS  
DOCUMENT NUMBER: PREV198733127633; BR33:127633  
TITLE: **STREPTOKINASE EXPRESSION OF ALTERED  
FORMS.**  
AUTHOR(S): MALKE H [Reprint author]; LORENZ D; FERRETTI J J  
CORPORATE SOURCE: ACAD SCI GER DEMOCRATIC REPUBLIC, CENT INST MICROBIOL AND  
EXP THERAPY, DDR-69 JENA, GDR  
SOURCE: (1987) pp. 143-149. FERRETTI, J. J. AND R. CURTISS, III  
(ED.). STREPTOCOCCAL GENETICS; SECOND ASM (AMERICAN SOCIETY  
FOR MICROBIOLOGY) CONFERENCE, MIAMI, FLORIDA, USA, MAY  
21-24, 1986. VIII+300P. AMERICAN SOCIETY FOR MICROBIOLOGY:  
WASHINGTON, D.C., USA. ILLUS.  
ISBN: 0-914826-93-X.

DOCUMENT TYPE: Book  
Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 27 Nov 1987  
Last Updated on STN: 27 Nov 1987

L17 ANSWER 26 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1987:277792 BIOSIS  
DOCUMENT NUMBER: PREV198784018831; BA84:18831  
TITLE: **MOLECULAR CLONING OF STREPTOKINASE GENE  
FROM STREPTOCOCCUS-EQUISIMILIS AND ITS**



EXPRESSION IN ESCHERICHIA-COLI.

AUTHOR(S): ROH D C [Reprint author]; KIM J H; PARK S K; LEE J W; BYRUN S M  
 CORPORATE SOURCE: DEP BIOLOGICAL SCIENCE AND ENGINEERING, KOREA ADVANCED INST SCIENCE AND TECHNOLOGY KAIST, PO BOX 150 CHONGRYANG, SEOUL 131, KOREA  
 SOURCE: Korean Biochemical Journal, (1986) Vol. 19, No. 4, pp. 391-398.  
 CODEN: KBCJAK. ISSN: 0368-4881.  
 DOCUMENT TYPE: Article  
 FILE SEGMENT: BA  
 LANGUAGE: ENGLISH  
 ENTRY DATE: Entered STN: 19 Jun 1987  
 Last Updated on STN: 19 Jun 1987

AB The streptococcal genomic DNA digested with Pst I was cloned in E. coli HB101. The overlay technique of casein/plasminogen was used to screen the clones for recombinants carrying the streptokinase gene. The insert size of the plasmid carrying the streptokinase gene was a 2.5, 4.3, and 5.8 Kb, respectively. The restriction maps of all three hybrid plasmids were constructed by digestion with Pst I, Pvu II, Sal I, Hind III, Ava I, BamH I, and Cla I. For the identification of cloned gene, streptokinase was highly purified from S. equisimilis by the methods of gel chromatography and isoelectric focusing and rabbits were immunized with this purified streptokinase. Several lines of evidence, including proof obtained by the immunodiffusion technique, established that the enzyme from E. coli was identical to that from S. equisimilis. In the E. coli cell culture, we found the activity of streptokinase in all three principal locations of the cell. More than 50% were existed in the intracellular space.

L17 ANSWER 27 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1984:100214 BIOSIS  
 DOCUMENT NUMBER: PREV198427016706; BR27:16706  
 TITLE: HYBRIDIZATION OF A CLONED GROUP C STREPTOCOCCAL STREPTO KINASE GENE WITH DNA FROM OTHER STREPTOCOCCAL SPECIES.  
 AUTHOR(S): HUANG T-T [Reprint author]; WEEKS C R; MALKE H; FERRETTI J J  
 CORPORATE SOURCE: UNIV OKLA HEALTH SCI CENT, OKLAHOMA CITY, OKLA, USA  
 SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D75.  
 Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.  
 ABSTR ANNU MEET AM SOC MICROBIOL.  
 CODEN: ASMAK. ISSN: 0094-8519.  
 DOCUMENT TYPE: Conference; (Meeting)  
 FILE SEGMENT: BR  
 LANGUAGE: ENGLISH

L17 ANSWER 28 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1984:92832 BIOSIS  
 DOCUMENT NUMBER: PREV198427009324; BR27:9324  
 TITLE: STREPTO KINASE CLONING EXPRESSION AND EXCRETION BY ESCHERICHIA-COLI.  
 AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J  
 CORPORATE SOURCE: ACAD SCI GDR, JENA, GDR  
 SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D97.  
 Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.  
 ABSTR ANNU MEET AM SOC MICROBIOL.

CODEN: ASMACK. ISSN: 0094-8519.

DOCUMENT TYPE: Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH

L17 ANSWER 29 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1994-07329 BIOTECHDS

TITLE: DNA encoding a plasminogen binding protein;  
recombinant streptokinase fragment  
production using new vector plasmid pMAL and a monoclonal  
antibody for use in myocardial infarction therapy

PATENT ASSIGNEE: Gen.Hosp.Boston; Univ.Harvard

PATENT INFO: WO 9407992 14 Apr 1994

APPLICATION INFO: WO 1993-US9502 5 Oct 1993

PRIORITY INFO: US 1993-128299 29 Sep 1993; US 1992-956692 5 Oct 1992

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1994-135561 [16]

AB DNA (I) encoding a **streptokinase** fragment (II) of residues 14-414 of a disclosed protein sequence is claimed. (II) does not contain residues 244-352, but may contain residues 1-352, 120-352, 244-414 or 244-352 of the protein sequence. Also claimed are: (1) an **expression** vector containing (I); (2) a host cell transformed with the vector of (1); (3) (II) encoded by (I); (4) detecting plasminogen in a biological sample by contacting the sample with (II) and detecting any (II)-plasminogen complex formed; (5) a method for assaying (II) for antigenicity involving contacting (II) with a monoclonal antibody specific for a distinct epitope of **streptokinase** and determining whether the fragments bind to the MAb and, optionally, also whether the fragments can activate plasminogen in the presence of the MAb; (6) a method for myocardial infarction therapy involving administering (II) to a patient; and (7) a monoclonal antibody specific for a distinct epitope of **streptokinase**. In an example, (II) genes from *Streptococcus equisimilis* were fused with maltose binding protein genes and **expressed** in *Escherichia coli* using plasmid pMAL. (62pp)

L17 ANSWER 30 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1992-13545 BIOTECHDS

TITLE: High level **expression** of **streptokinase** in  
*Escherichia coli*;

gene cloning, **expression** and  
purification of thrombolytic protein

AUTHOR: Estrada M P; Hernandez L; Perez A; Rodriguez P; \*de la Fuente  
J; Herrera L

LOCATION: Mammalian Cell Genetics Division, Centro de Ingenieria  
Genetica y Biotecnologia, P.O. Box 6162, Havana 6, Cuba.

SOURCE: Bio/Technology; (1992) 10, 10, 1138-42  
CODEN: BTCHDA

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **streptokinase** (SK) gene was isolated by the polymerase chain reaction from *Streptomyces equisimilis* ATCC 9542. The 5-amplification primer introduced an ATG codon for translation initiation in *Escherichia coli*. The amplified fragment, which lacked the signal peptide sequence, was digested with BamHI, inserted into vector plasmid pTrp (to obtain plasmid pEKG-3 containing the SK gene under the control of a trp promoter), and used to transform *E. coli* HB101 cells. The DNA sequence of the SK gene region contained 5 differences at the amino acid level with respect to the reported SK protein. Plasmid pEKG-3 was introduced into *E. coli* K-12 strain W3110 for **expression**. The trp promoter was induced, and maximal SK **expression** was obtained after 14 hr, at which time the plasmid copy number reached 420 copies/cell. The **recombinant** SK was

found in the cell cytosol and constituted 25% of total cell protein. It was purified by affinity chromatography using acylated human plasminogen coupled to Sepharose-4B, and ionexchange chromatography on DEAE-Sephacel. The **recombinant** product and natural SK had equivalent biological activities. (38 ref)

L17 ANSWER 31 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1992-03808 BIOTECHDS

TITLE: Method for the isolation and **expression** of a gene encoding **streptokinase**;  
Streptococcus **equisimilis** gene cloning  
and vector plasmid pEKG3, plasmid pPESKC-4 and plasmid pPISKC-6 **expression** in Escherichia coli  
or Pichia pastoris

PATENT ASSIGNEE: Cent.Ing.Genet.Biotechnol.

PATENT INFO: AU 9178101 28 Nov 1991

APPLICATION INFO: AU 1991-78101 31 May 1991

PRIORITY INFO: CU 1990-90 23 May 1990

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1992-024716 [04]

AB A new method for the isolation and **expression** of a gene, **SKC-2**, encoding Streptococcus **equisimilis** C (ATCC 9542) **streptokinase** comprises (i) gene amplification from synthetic oligonucleotides SK1, SK2 and SK3 (specified DNA sequence), (ii) **cloning** **SKC-2** in bacteria (preferably Escherichia coli) with or without a signal peptide; and (iii) intra- or extracellular **expression** in yeast (preferably Pichia pastoris), with the transformed microorganism displaying a high stability and level of **expression**. The following are also claimed: (1) plasmid pEKG3 containing **SKC-2** inserted between the trp promoter and the phage T4 terminator for **expression** in bacteria; (2) plasmid pPESKC-4 and plasmid pPISKC-6, obtained by insertion of **SKC-2** in the yeast **expression** vectors plasmid pPS-7 and plasmid pNAO, respectively, for extra- or intracellular **expression**; (3) transformed microorganisms displaying high levels of **SKC-2** gene **expression**, good viability and cellular stability; (4) the product resulting from **expression** of the **SKC-2** gene in bacteria and yeast; (5) **recombinant** DNA comprising the **SKC-2** DNA sequence; and (6) the **expression** product of the **recombinant** DNA. (28pp)

L17 ANSWER 32 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1991-01878 BIOTECHDS

TITLE: Constructing vector for detecting **expression** of foreign genes;  
by inserting element containing **expression** unit, **streptokinase** gene and restriction sites, allowing in frame gene insertion; pro-chymosin, beta-galactosidase production

PATENT ASSIGNEE: Akad.Wiss.DDR

PATENT INFO: DD 279900 20 Jun 1990

APPLICATION INFO: DD 1987-306609 3 Sep 1987

PRIORITY INFO: DD 1987-306609 3 Sep 1987

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 1990-342373 [46]

AB Construction of vectors for detecting heterologous gene **expression** comprises: i. incorporating (in order from N terminus) into a **cloning** vector, a polylinker or restriction site (RS1); **expression** or **expression** secretion unit (Escherichia coli lac operon or exotoxin A gene of phage T12 from Streptococcus pyogenes; polylinker or restriction site (RS2);

**streptokinase** (SK) structural gene (from *Streptococcus equisimilis* H46A, particularly a 1596 bp HindIII fragment from plasmid pMF5), a polylinker or restriction site (RS3); ii. inserting a foreign gene, X, without a promoter into polylinker or RS2; iii. the resulting detection vector, encoding for an X-SK fusion product, is used to transform microbial receptor cells; and iv. subjecting **recombinant clones** to a plasminogen-milk agar (PMA) overlaying test. Preferably, the vector is a bacterial plasmid or an M13 *E. coli* phage vector. X is a pro-chymosin gene (plasmid pHRW400 or plasmid pHRW500), human interferon-alpha-1, or beta-galactosidase (EC-3.2.1.23, from a pUC plasmid). Recipients are *E. coli* JM101, *Streptococcus sanguis* Challis 6 or *Streptococcus lactis* MG1363. (14pp)

L17 ANSWER 33 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1990-02600 BIOTECHDS

TITLE: Site-specific alteration of Gly-24 in **streptokinase**  
: its effect on plasminogen activation;  
site-directed mutagenesis effect on plasminogen-activator  
activity; gene cloning and **expression**  
in *Escherichia coli*

AUTHOR: Lee B R; Park S K; Kim J H; \*Byun S M

LOCATION: Department of Biological Science and Engineering, Korea  
Advanced Institute of Science and Technology (KAIST), P.O.  
Box 150, Cheongryang, Seoul, Korea.

SOURCE: Biochem.Biophys.Res.Commun.; (1989) 165, 3, 1085-90

CODEN: BBRC A9

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Oligonucleotide site-directed mutagenesis was performed to replace Gly-24 of *Streptococcus equisimilis* (ATCC 9542) **streptokinase** with His, Glu, or Ala. The **streptokinase** gene was **cloned**, subjected to mutagenesis for removal of the RsaI site, **cloned** into vector plasmid pKS601 under the control of the trp promoter and used to transform *Escherichia coli* C600. The **recombinant** proteins were purified by DEAE-cellulose and Sephadex-G150 chromatography. Substitutions with either His or Glu gave almost complete loss of **streptokinase** activity but **streptokinase** replaced with Ala retained its activity. Although **streptokinases** with His-24 or Glu-24 bound normally to human plasminogen, they did not generate active plasmin, whereas those with Ala-24 or Gly-24 generated active plasmin. The results indicate that the small, uncharged alkyl group side chain on the 24th amino acid residue of **streptokinase** is indispensable for the activity of the human plasminogen-**streptokinase** complex. A charged amino acid in position 24 disrupts beta-sheet formation and prevents **streptokinase** from adopting the orientation required for plasminogen activation. (26 ref)

L17 ANSWER 34 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1985-08513 BIOTECHDS

TITLE: Production of **streptokinase**;  
by cultivation of *Escherichia coli* ATCC 39613  
containing **recombinant** plasmid pMF1

PATENT ASSIGNEE: Phillips-Petrol.

PATENT INFO: AU 8433859 18 Apr 1985

APPLICATION INFO: AU 1984-33859 5 Oct 1984

PRIORITY INFO: US 1984-585417 2 Mar 1984; DD 1983-255523 10 Oct 1983

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1985-135032 [23]

AB A **recombinant** vector plasmid pMF1 for the transformation of a host to produce **streptokinase** is new. The vector contains a polydeoxyribonucleotide fragment insert which codes for the synthesis and

secretion of **streptokinase**. The transformant microorganism is preferably *Escherichia coli* HB101 and the vector, plasmid pBR322. The fragment coding for **streptokinase** synthesis and secretion is derived from a microorganism of the genus *Streptococcus*, especially *Streptococcus equisimilis* strain H46A and may have restriction endonuclease cleavage sites at the termini e.g. it has 7400 bp and the cleavage sites are for HindIII. The **recombinant** vector is obtained by digestion of a vector with a restriction endonuclease to give linear DNA. This DNA is ligated to the **streptokinase** fragment to give the **recombinant**. This fragment is obtained by digestion of *Streptococcus equisimilis* with the same restriction endonuclease, especially Pst I, as is used to digest the initial vector. **Streptokinase** can be produced and isolated for use as a thrombolytic agent to facilitate the in vivo lysis or dissolution of blood clots. (28pp)

L17 ANSWER 35 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1984-03868 BIOTECHDS

TITLE: **Streptokinase: cloning expression**  
and excretion by *E.coli*;  
using *Streptococcus equisimilis* genomic DNA  
(conference abstract)

AUTHOR: Malke H; Ferretti J J

LOCATION: Acad. Sci. GDR, Jena, DDR.

SOURCE: Abstr.Annu.Meet.Am.Soc.Microbiol; (1984) 84 Meet., 67

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Genomic DNA from *Streptococcus equisimilis* H46A (group C) was cloned into *Escherichia coli* using the lambda replacement vector L47 and an in vitro packaging system. The phage bank was screened for **recombinants** containing the **streptokinase (skc)** gene by the casein-plasminogen overlay technique. 10 Independent clones containing the **skc** gene were isolated and one was used to subclone the **skc** gene into *E.coli* plasmid vectors pBR322 and pACYC184. Plasmid pMF2 and pMF5 were among the **recombinant** plasmids determining **streptokinase** production in 3 different *E. coli* host strains. Expression of **skc** was independent of its orientation in either vector, indicating that its own promoter was present and functional in *E.coli*. Analysis of cell free culture supernatant fluids, osmotic shockates, and sonicates of osmotically shocked cells for **streptokinase** activity revealed the substance to be present in all 3 locations, indicating that *E. coli* cells were capable of releasing substantial amounts of **streptokinase** into the culture medium. (0 ref)

L17 ANSWER 36 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:656911 SCISEARCH

THE GENUINE ARTICLE: 837ZE

TITLE: Control of **streptokinase** gene expression  
in group A & C streptococci by two-component regulators

AUTHOR: Malke H (Reprint); Steiner K

CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany  
hmalke@imb-jena.de

COUNTRY OF AUTHOR: Germany

SOURCE: INDIAN JOURNAL OF MEDICAL RESEARCH, (MAY 2004) Vol. 119, Supp. [S], pp. 48-56.  
ISSN: 0971-5916.

PUBLISHER: INDIAN COUNCIL MEDICAL RES, PO BOX 4911 ANSARI NAGAR, NEW DELHI 110029, INDIA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 23

ENTRY DATE: Entered STN: 13 Aug 2004

Last Updated on STN: 13 Aug 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Background & objectives: Group A streptococci (GAS) and human isolates of group C streptococci (GCS) have the stable capacity to produce the plasminogen activator **streptokinase**, albeit with varying efficiency. This property is subject to control by two two-component regulatory systems, FasCAX and CovRS, which act as activator and repressor, respectively. The present work aims at balancing these opposing activities in GAS and GCS, and at clarifying the phylogenetic position of the FasA response regulator, the less understood regulator of the two systems.

Methods: The GCS strain H46A and GAS strain NZ131 were used. *Escherichia coli* JM 109 was used as host for plasmid construction. **Streptokinase** activity of various wild type and mutant strains was measured. Phylogenetic trees of streptococcal FasA homologues were established.

Results: The **streptokinase** activities of the GAS strain NZ131 and the GCS strain H46A were attributable to more efficient CovR repressor action in NZ131 than in H46A. The FasA activator, on the other hand, functioned about equally efficient in the two strains. Phylogenetically, FasA homologues clustered distinctly in the proposed FasA-BlpR-ComE family of streptococcal response regulators and used the LytTR domain for DNA binding.

Interpretation & conclusion: Assessing the apparent **streptokinase** activity of streptococcal strains require the dissection of the activities of the cov and fas systems. Although experimental evidence is still missing, FasA is closely related to a widely distributed family of streptococcal response regulators that is involved in behavioral processes, such as quorum sensing.

L17 ANSWER 37 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:69727 SCISEARCH

THE GENUINE ARTICLE: 763KK

TITLE: **Streptokinase** - a clinically useful thrombolytic agent

AUTHOR: Banerjee A; Chisti Y; Banerjee U C (Reprint)

CORPORATE SOURCE: Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Sector 67, Mohali 160062, Punjab, India (Reprint); Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Mohali 160062, Punjab, India; Massey Univ, Inst Technol & Engrn, Palmerston North, New Zealand

COUNTRY OF AUTHOR: India; New Zealand

SOURCE: BIOTECHNOLOGY ADVANCES, (FEB 2004) Vol. 22, No. 4, pp. 287-307.

ISSN: 0734-9750.

PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.

DOCUMENT TYPE: General Review; Journal

LANGUAGE: English

REFERENCE COUNT: 171

ENTRY DATE: Entered STN: 30 Jan 2004

Last Updated on STN: 30 Jan 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A failure of hemostasis and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infarction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and **streptokinase**. This review deals with **streptokinase** as a clinically important and cost-effective plasminogen activator. The aspects discussed include: the mode of action;

the structure and structure-function relationships; the structural modifications for improving functionality; **recombinant streptokinase**; microbial production; and recovery of this protein from crude broths. (C) 2003 Published by Elsevier Inc.

L17 ANSWER 38 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:148580 SCISEARCH  
THE GENUINE ARTICLE: 285UW  
TITLE: Allele substitution of the **streptokinase** gene reduces the nephritogenic capacity of group A streptococcal strain NZ131  
AUTHOR: Nordstrand A (Reprint); McShan W M; Ferretti J J; Holm S E; Norgren M  
CORPORATE SOURCE: Umea Univ, Dept Clin Bacteriol, S-90185 Umea, Sweden (Reprint); Univ Oklahoma, Hlth Sci Ctr, Dept Microbiol & Immunol, Oklahoma City, OK 73190 USA  
COUNTRY OF AUTHOR: Sweden; USA  
SOURCE: INFECTION AND IMMUNITY, (MAR 2000) Vol. 68, No. 3, pp. 1019-1025.  
ISSN: 0019-9567.  
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 36  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB To investigate the role of allelic variants of **streptokinase** in the pathogenesis of acute poststreptococcal glomerulonephritis (APSGN), site-specific integration plasmids were constructed, which contained either the non-nephritis-associated **streptokinase** gene (skc5) from the group C streptococcal strain *Streptococcus equisimilis* H46A or the nephritis-associated **streptokinase** gene (ska1) from the group A streptococcal nephritogenic strain NZ131. The plasmids were introduced by electroporation and homologous recombination into the chromosome of an isogenic derivative of strain NZ131, in which the **streptokinase** gene had been deleted and which had thereby lost its nephritogenic capacity in a mouse model of APSGN. The introduction of a non-nephritis-associated allelic variant of **streptokinase** did not rescue the nephritogenic capacity of the strain. The mutant and the wild-type strains produced equivalent amounts of **streptokinase**. Complementation of the ska deletion derivative with the original ska allele reconstituted the nephritogenicity of wild-type NZ131. The findings support the hypothesis that the role of **streptokinase** in the pathogenesis of APSGN is related to the allelic variant of the protein.

L17 ANSWER 39 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:100732 SCISEARCH  
THE GENUINE ARTICLE: 277JH  
TITLE: Genetic organisation of the M protein region in human isolates of group C and G streptococci: two types of multigene regulator-like (mgrC) regions  
AUTHOR: Geyer A; Schmidt K H (Reprint)  
CORPORATE SOURCE: Univ Jena, Univ Hosp, Inst Med Microbiol, Semmelweisstr 4, D-07740 Jena, Germany (Reprint); Univ Jena, Univ Hosp, Inst Med Microbiol, D-07740 Jena, Germany  
COUNTRY OF AUTHOR: Germany  
SOURCE: MOLECULAR AND GENERAL GENETICS, (JAN 2000) Vol. 262, No. 6, pp. 965-976.  
ISSN: 0026-8925.

PUBLISHER: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 47  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In addition to beta-haemolytic streptococci belonging to Lancefield group A (*Streptococcus pyogenes*, GAS), human isolates of group C (GCS) and group G (GGS) streptococci (*S. dysgalactiae* subsp. *equisimilis*;) have been implicated as causative agents in outbreaks of purulent pharyngitis, of wound infections and recently also of streptococcal toxic shock-like syndrome. Very little is known about the organisation of the genomic region in which the emm gene of GCS and GCS is located. We have investigated the genome sequences flanking the emm gene in GCS by sequencing neighbouring fragments obtained by inverse PCR. Our sequence data for GCS strains 25287 and H46A revealed two types of arrangement in the emm region, which differ significantly from the known types of mga regulon in GAS. We named this segment of the genome mgrC (for multigene regulon-like segment in group C streptococci). In strains belonging to the first mgrC type (prototype strain 25287) the emm gene is flanked upstream by mgc, a gene that is 61% identical to the mga gene of GAS. A phylogenetic analysis of the deduced protein sequences showed that Mgc is related to Mga proteins of various types of GAS but forms a distinct cluster. Downstream of emm, the mgrC sequence region is bordered by rel. This gene encodes a protein that functions in the synthesis and degradation of guanosine 3',5' bipyrophosphate (ppGpp) during the stringent regulatory response to amino acid deprivation. In the second mgrC type (prototype strain H46A), the genes mgc and emm are arranged as in type 1. But an additional ORF (orf) is inserted in opposite orientation between emm and rel. This orf shows sequence homology to cpdB, which is present in various microorganisms and encodes 2',3' cyclo-nucleotide 2'-phosphodiesterase. PCR analysis showed that these two mgrC arrangements also exist in GGS. Our sequence and PCR data further showed that both types of mgrC region in GCS and GGS are linked via rel to the **streptokinase** region characterised recently in strain H46A. A gene encoding C5a peptidase, which is present at the 3' end of the mga regulon in GAS, was not found in the mgrC region identified in the GCS and GGS strains investigated here.

L17 ANSWER 40 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:666643 SCISEARCH  
THE GENUINE ARTICLE: 231GM  
TITLE: PauA: a novel plasminogen activator from *Streptococcus uberis*  
AUTHOR: Rosey E L; Lincoln R A; Ward P N; Yancey R J; Leigh J A (Reprint)  
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint); Pfizer Inc, Div Cent Res, Anim Hlth Biol Discovery, Groton, CT 06340 USA  
COUNTRY OF AUTHOR: England; USA  
SOURCE: FEMS MICROBIOLOGY LETTERS, (1 SEP 1999) Vol. 178, No. 1, pp. 27-33.  
ISSN: 0378-1097.  
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 20  
ENTRY DATE: Entered STN: 1999  
Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Chromosomal DNA from two geographically distinct isolates of



Streptococcus uberis was used to clone the plasminogen activator in an active form in Escherichia coli. The cloned fragments from each strain contained four potential open reading frames (ORFs). That for the plasminogen activator encoded a protein of 286 amino acids (33.4 kDa) which is cleaved between residues 25 and 26 during secretion by S. uberis. The amino acid sequence of the mature protein showed only weak homology (23.5-28%) to streptokinase. The plasminogen activator gene, pauA, in S. uberis was located between two ORFs with high homology to the DNA mismatch repair genes, hexA and hexB, and not on a DNA fragment between the genes encoding an ATP binding cassette transporter protein (abc) and a protein involved in the formation and degradation of guanosine polyphosphates (rel) as is the case for streptokinase in other streptococci. (C) 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

L17 ANSWER 41 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:317401 SCISEARCH

THE GENUINE ARTICLE: 189BZ

TITLE: Hydrophobic interaction chromatography applied to purification of recombinant streptokinase

AUTHOR: Perez N (Reprint); Urrutia E; Camino J; Orta D R; Torres Y; Martinez Y; Cruz M; Alburquerque S; Gil M R; Hernandez L

CORPORATE SOURCE: Ctr Genet Engn & Biotechnol, Streptokinase Div, Havana, Cuba; Ctr Genet Engn & Biotechnol, Qual Control Div, Havana, Cuba

COUNTRY OF AUTHOR: Cuba

SOURCE: MINERVA BIOTECNOLOGICA, (DEC 1998) Vol. 10, No. 4, pp. 174-177.  
ISSN: 1120-4826.

PUBLISHER: EDIZIONI MINERVA MEDICA, CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 22

ENTRY DATE: Entered STN: 1999

Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Background Recombinant streptokinase (rSk) is a streptococcal protein cloned in E. coli.(11) Several methods have been described for streptokinase purification: ion exchange chromatography,(12) affinity chromatography with canine plasmin(13) and chromatography on immobilized acylated human plasminogen.(14) Monoclonal antibodies anti-rSk immobilized to Sepharose(15) have been used too. Recently this protein was purified using HIC.

Methods. rSk (CIGB, Cuba) was produced by fermentation strain K12 of E. coli,(11) the protein was recovered after washed pellet, cellular disruption and solubilization. Several purification assays were done using TSK-Butyl (Tosohaas, Japan) as a support for hydrophobic interaction chromatography (HIC). The protein was loaded to the column with 1 M of ammonium sulfate before being washed using an elution gradient from 0.5 to 0 M of ammonium sulfate, in order to determine the elution point of the rSk.

Results. Wie could determine that this protein is partly hydrophobic, this determination was shown by analysis of its aminoacidic sequence. This protein has 415 aminoacids of which 36% are non polar. The absorption capacity for TSK Butyl 650 S varies from 15 to 20 mg/mL. The optimum elution point was obtained using 0.25 M of ammonium sulfate, the eluted material was obtained with a high level of purity (<1% of contaminants). The recovery of rSk was about 49% using the mean of five

assays.

Conclusions. The experimental process evaluated could be efficiently inserted in a downstream process to obtain **recombinant streptokinase** highly purified as final preparation and good recovery.

L17 ANSWER 42 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:473042 SCISEARCH

THE GENUINE ARTICLE: ZV077

TITLE: The interaction of *Streptococcus dysgalactiae* with plasmin and plasminogen

AUTHOR: Leigh J A (Reprint); Hodgkinson S M; Lincoln R A

CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint)

COUNTRY OF AUTHOR: England

SOURCE: VETERINARY MICROBIOLOGY, (15 MAR 1998) Vol. 61, No. 1-2, pp. 121-135.

ISSN: 0378-1135.

PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 29

ENTRY DATE: Entered STN: 1998

Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The activation of plasminogen and the binding of plasmin by bacteria may have many effects which promote infection. The occurrence of such activities in streptococci is well documented; however, these are yet to be demonstrated for *S. dysgalactiae*. Consequently, the ability of this bacterium to activate mammalian plasminogen and bind either plasmin or its zymogen was investigated. Activation of bovine plasminogen was dependant on both the strain and the growth medium used for cultivation. Eighteen strains were able to activate bovine and ovine plasminogen and some of these also activated plasminogen from the horse, rabbit and pig. None activated human plasminogen and one strain (CE127) did not activate plasminogen from any source. Tricine-SDS PAGE and zymographic analysis of culture supernatants showed that bovine plasminogen was activated by four out of six strains at two locations corresponding to 16 kDa and 10 kDa. Following the growth of five strains in the presence of bovine plasminogen, all but strain CE127 bound high levels of plasmin activity. In contrast, following growth in human plasminogen none of the strains exhibited bound plasmin activity although all could bind human plasmin directly. All strains were also able to bind bovine and human plasminogen in such a way as to allow its activation by urokinase. We conclude that *S. dysgalactiae* is capable of activating mammalian plasminogen in a species-specific fashion and that the bacterium is also capable of binding plasmin and plasminogen with an apparent preference for bovine plasmin over human plasmin and/or plasminogen from either species. (C) 1998 Elsevier Science B.V.

L17 ANSWER 43 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:437021 SCISEARCH

THE GENUINE ARTICLE: ZU066

TITLE: The *Streptococcus agalactiae* hylB gene encoding hyaluronate lyase: completion of the sequence and **expression** analysis

AUTHOR: Gase K; Ozegowski J; Malke H (Reprint)

CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany; Univ Jena, Inst Expt Microbiol, D-07745 Jena, Germany

COUNTRY OF AUTHOR: Germany  
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND  
EXPRESSION, (29 MAY 1998) Vol. 1398, No. 1, pp. 86-98.  
ISSN: 0167-4781.  
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
NETHERLANDS.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 43  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We report the **cloning**, sequencing and **expression** analysis of the *Streptococcus agalactiae* strain 4755 hylB(4755) allele, the first chromosomally-encoded streptococcal hyaluronate lyase gene to be **cloned** and sequenced completely. This gene lies in a region homologous to that found in *S. mutans*, between the mutX and rmlB genes, a region involved in the synthesis of the serotype c-specific polysaccharide antigen of this organism. Sequencing of hylB(4755) revealed a 3216-bp open reading frame that encodes a 121.2-kDa polypeptide possessing a 30-amino acid signal sequence which was theoretically predicted and experimentally confirmed. A **recombinant** plasmid, pHYB100, containing hylB(4755) together with its promoter and terminator was constructed and used to analyze the **expression** of the gene in *Escherichia coli*. In Northern hybridization experiments, hylB(4755) was found to be transcribed as 3.3-kb monocistronic mRNA from its own promoter which exhibits an extended, sigma(70)-like 10 consensus sequence. Transcript mapping by primer extension analysis placed the major transcription initiation site leading to the longest transcript 38 bp upstream of the translational initiation codon: ATG. *E. coli* TG1(pHYB100) efficiently synthesized hyaluronan-cleaving enzyme activity at similar to 7000 working units/10(9) cells, with lyase activity detectable in all principle cellular locations. Zymography and Western analysis identified functional activity in TG1(pHYB100) to be associated with similar to 118, 110 and 94-kDa polypeptides, with the two low molecular weight species constituting the major components of the enzyme purified from the culture supernatant fluid of *S. agalactiae* 4755. The 118-kDa form was shown to represent the undegraded mature enzyme, whereas the smaller species are likely to arise from proteolytic cleavage in the N-terminal part of the mature protein. The HylB(4755) protein showed extensive sequence identity to the homologous enzymes from *S. agalactiae* 3502 and *S. pneumoniae* characterized by others but sequence comparisons clearly show that incomplete genes truncated at their 5' ends had been isolated from these two organisms. (C) 1998 Elsevier Science B.V. All rights reserved.

L17 ANSWER 44 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:547160 SCISEARCH  
THE GENUINE ARTICLE: XL484  
TITLE: The LppC gene of *Streptococcus equisimilis* encodes a lipoprotein that is homologous to the e(P4) outer membrane protein from *Haemophilus influenzae*  
AUTHOR: Gase K (Reprint); Liu G W; Bruckmann A; Steiner K; Ozegowski J; Malke H  
CORPORATE SOURCE: UNIV JENA, INST MOL BIOL, D-07745 JENA, GERMANY  
COUNTRY OF AUTHOR: GERMANY  
SOURCE: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (JUN 1997) Vol. 186, No. 1, pp. 63-73.  
ISSN: 0300-8584.  
PUBLISHER: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English

REFERENCE COUNT: 39

ENTRY DATE: Entered STN: 1997

Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We report the cloning, sequencing, and analysis of a novel chromosomal gene of *Streptococcus equisimilis* strain H46A that codes for a membrane lipoprotein, designated LppC. The lppC gene is located 3' adjacent to, and co-oriented with, the unrelated gapC gene that encodes the previously characterized glyceraldehyde-3-phosphate dehydrogenase. Sequencing of lppC revealed an 855-bp open reading frame that predicted a 32.4-kDa polypeptide possessing a potential lipoprotein signal sequence and modification site (VTGC). Signal sequence processing of LppC synthesized in the homologous host or expressed from plasmid pLPP2 in *Escherichia coli* was sensitive to globomycin, a selective inhibitor of lipoprotein-specific signal peptidase II. Subcellular localization of LppC using polyclonal antibodies raised to the hexahistidyl-tagged protein proved LppC to be tightly associated with the cytoplasmic membrane of *S. equisimilis* and with the outer membrane of *E. coli* JM109 (pLPP2). Southern, Northern and Western analyses indicated that Ipl, was conserved in *S. pyogenes*, and transcribed independently of gap as monocistronic 0.9-kb mRNA from a sigma(70)-like consensus promoter. Database searches found homology of LppC to the hel gene-encoded outer membrane protein e (P4) from *Haemophilus influenzae* to which it exhibits 58% sequence similarity. However, unlike the hel gene, lppC was unable to complement hemA mutants of *E. coli* for growth on hemin as sole porphyrin source in aerobic conditions. Furthermore, neither the wild type nor an lppC insertion mutant of *S. equisimilis* could grow on hemin in iron-limited medium. These results, together with findings indicating that *S. equisimilis* H46A had no absolute requirement for iron, led us to conclude that lppC, in contrast to hel, is not involved in hemin utilization and has yet to be assigned a function.

L17 ANSWER 45 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1996:43901 SCISEARCH

THE GENUINE ARTICLE: TN446

TITLE: Cloning and sequencing of the streptokinase gene from *Streptococcus pyogenes* (CIP 56.57)

AUTHOR: Ball M M (Reprint); Puig J; Iborra F

CORPORATE SOURCE: UNIV PARIS 11, F-91405 ORSAY, FRANCE

COUNTRY OF AUTHOR: FRANCE

SOURCE: DNA SEQUENCE, (1995) Vol. 6, No. 1, pp. 33-36.  
ISSN: 1042-5179.

PUBLISHER: HARWOOD ACAD PUBL GMBH, C/O STBS LTD, PO BOX 90, READING, BERKS, ENGLAND RG1 8JL.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 10

ENTRY DATE: Entered STN: 1996

Last Updated on STN: 1996

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The streptokinase gene of the *Streptococcus pyogenes* strain CIP 56.57 was cloned and sequenced. This sequence coding for a 441 amino acid protein is well conserved among streptococcus species: there are two very conserved domains separated by a more variable region.

L17 ANSWER 46 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1995:622543 SCISEARCH

THE GENUINE ARTICLE: RU780

TITLE: STREPTOKINASE-MEDIATED PLASMINOGEN ACTIVATION

USING A RECOMBINANT DUAL FUSION PROTEIN  
CONSTRUCT - A NOVEL-APPROACH TO STUDY BACTERIAL HOST  
PROTEIN INTERACTIONS

AUTHOR: LIZANO S (Reprint); JOHNSTON K H  
CORPORATE SOURCE: LOUISIANA STATE UNIV, MED CTR, DEPT MICROBIOL IMMUNOL &  
PARASITOL, NEW ORLEANS, LA 70112  
COUNTRY OF AUTHOR: USA  
SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (SEP 1995) Vol. 23,  
No. 3, pp. 261-280.  
ISSN: 0167-7012.  
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
NETHERLANDS.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 59  
ENTRY DATE: Entered STN: 1995  
Last Updated on STN: 1995

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Streptokinase (SK), a plasminogen (Pg) activator secreted by  
groups A, C, and G streptococci, is extensively used as a pharmacological  
agent in thrombolytic therapy and possibly plays a role in streptococcal  
invasiveness and disease. SK activates Pg to plasmin (Ps) by forming an  
activator complex with Pg. However, the molecular basis whereby SK binds  
and activates Pg remains unclear, in part due to the rapid fragmentation  
of the SK-Pg complex. This study describes a solid phase approach to  
study this interaction in which a recombinant SK molecule was  
constructed with glutathione-S-transferase appended to the NH2 terminus  
and (Gly)(3)(His)(8) appended to the COOH terminus. This dual fusion  
protein molecule, immobilized on either Sepharose-S-hexylglutathione or  
Ni2+ dinitriloacetic acid-Sepharose was then used to study the interaction  
of SK with Pg. These SK-Pg complexes exhibited amidolytic and proteolytic  
activity similar to native SK, but the pattern of fragmentation of the SK  
molecule was dependent upon whether the SK molecule was immobilized either  
at its NH2- or COOH terminus. This solid phase approach may contribute to  
a greater understanding of the role of SK in Pg activation by enabling the  
'capture' of intact activator complexes under physiological conditions  
and, in addition, may serve as a useful model to analyze other  
bacterial-host protein interactions important in the pathogenesis of  
disease.

L17 ANSWER 47 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:29440 HCAPLUS  
DOCUMENT NUMBER: 142:428913  
TITLE: An improved process for the simultaneous preparation  
of extracellular streptokinase and its  
analogues  
INVENTOR(S): Dikshit, Kanak Lata; Vyas, Vinay Venkatrao; Mahajan,  
Ritu; Kaur, Jaodeep; Thapar, Nitika; Phatap, Jitesh;  
Nihalani, Deepak; Sahni, Girish  
PATENT ASSIGNEE(S): Council of Scientific and Industrial Research, India  
SOURCE: Indian, 122 pp.  
CODEN: INXXAP  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IN 183828	A	20000429	IN 1994-DE1727	19941230
PRIORITY APPLN. INFO.:			IN 1994-DE1727	19941230

AB Extracellular streptokinase and its analogs prepared by growing  
recombinant E.coli in a conventional fermentation medium under

stirring and supplemented with aeration, separating the cells from supernatant by known methods followed by recovering and purifying Streptokinase and its analogs from supernatant.

L17 ANSWER 48 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:175570 HCAPLUS  
DOCUMENT NUMBER: 132:218864  
TITLE: Streptokinase analogs with low antigenicity  
for use as thrombolytics  
INVENTOR(S): Torrens Madrazo, Isis Del Carmen; Garcia Ojalvo,  
Ariana; De La Fuente Garcia, Jose De Jesus; Seralena  
Menendez, Alina  
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB),  
Cuba  
SOURCE: Eur. Pat. Appl., 54 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 985729	A2	20000315	EP 1999-202639	19990813
EP 985729	A3	20000531		
EP 985729	B1	20050427		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9943424	A1	20000309	AU 1999-43424	19990805
AU 769916	B2	20040212		
CA 2277554	C	20041102	CA 1999-2277554	19990806
CA 2277554	AA	20000214		
US 6309873	B1	20011030	US 1999-374038	19990813
AT 294239	E	20050515	AT 1999-202639	19990813
US 6413759	B1	20020702	US 2000-658179	20000908
PRIORITY APPLN. INFO.:			CU 1998-119	A 19980814
			US 1999-374038	A3 19990813

AB Streptokinase analogs with antigenic domains modified to minimize antigenicity are described for use in the treatment of clotting-associated disorders. The proteins retain their capacity for plasminogen activator complex formation. The proteins are manufactured by expression of the corresponding allele of the skc2 gene encoding streptokinase SKC-2 (Heberkinase®). The mols. obtained from present invention can be used in the treatment of disorders as myocardial infarction, pulmonary thromboembolism, surgical complications and other cases of thrombosis.

L17 ANSWER 49 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:579938 HCAPLUS  
DOCUMENT NUMBER: 131:183948  
TITLE: Manufacture of streptokinase for therapeutic  
use by expression of the cloned  
gene in Pichia pastoris  
INVENTOR(S): Estrada Garcia, Mario; Rubiera Chaplen, Roger; Perez,  
Hidalgo; Serrano Doce, Ricardo; Hernandez Marrero,  
Luciano F.; Rodriguez Collazo, Pedro; Castro Ramirez,  
Anaisel; Munoz Munoz, Emilio Amable; Bravo Martinez,  
Walfrido; Campos Somavilla, Magalys; Pedraza  
Fernandez, Alicia; De la Furente Garcia, Jose de J.;  
Herrera Martinez, Luis S.  
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB),  
Cuba  
SOURCE: Czech Rep., 18 pp.  
CODEN: CZXXED

DOCUMENT TYPE: Patent  
 LANGUAGE: Czech  
 FAMILY ACC. NUM. COUNT: 2  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CZ 284692	B6	19990217	CZ 1991-2256	19910719
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A 19900523
			CS 1991-2256	A 19910719
			SU 1991-5001280	A 19910717

AB **Streptokinase** for therapeutic use is manufactured by expression of the cloned gene in *Pichia pastoris*. The protein may be secreted into the culture medium or accumulated intracellularly. The gene was cloned from a type C *Streptococcus equisimilis* by PCR. Expression of the gene from the AOX1 promoter using *Pichia pastoris* as the host resulted in the manufacture of an enzyme with a specific activity of 50,000-100,000 fibrin-agarose units/mg protein.

L17 ANSWER 50 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN  
 ACCESSION NUMBER: 1999:405093 HCAPLUS  
 DOCUMENT NUMBER: 131:54027  
 TITLE: Fibrin-dependent plasminogen activator activity of modified bacterial **streptokinases**  
 INVENTOR(S): Reed, Guy L.  
 PATENT ASSIGNEE(S): The President and Fellows of Harvard College, USA  
 SOURCE: PCT Int. Appl., 73 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9931247	A1	19990624	WO 1998-US26694	19981215
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9918295	A1	19990705	AU 1999-18295	19981215
US 6210667	B1	20010403	US 1998-211542	19981215
PRIORITY APPLN. INFO.:			US 1997-69497P	P 19971215
			WO 1998-US26694	W 19981215

AB A pharmaceutical composition in a preferred embodiment comprises an isolated bacterial protein **streptokinase** that induces fibrin-dependent plasminogen activation, and methods for dissolving blood clots in a subject using such a composition. Two preferred **streptokinase** mutants and truncated derivs. comprising residues 144-293 and residues 60-414 of the *Streptococcus equisimilis* H46A enzyme. Deletion of the first 59 amino acids to product mutant rSK60-414 yielded a protein with a 767-fold decrease in kcat compared to that of rSK1-414, without any significant change in the Km. The N-terminus dets. the clot (fibrin) dependence of plasminogen activation by **streptokinase** and the regulation of plasminogen activation in the presence of fibrin. By virtue of its requirement for fibrin for plasminogen activation in human plasma, and its sparing of fibrinogen during clot dissoln., **streptokinase** deleted of N-terminal amino acid residues is similar to tissue-type

plasminogen activator. Embodiments also include a nucleic acid encoding such as a bacterial protein, a nucleic acid encoding such a bacterial protein as a fusion to another protein, an **expression** vector with the nucleic acid, and a host cell transformed with the **expression** vector.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 51 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1997:155090 HCAPLUS

DOCUMENT NUMBER: 126:154444

TITLE: **Streptokinases** analogs resistant to cleavage and inactivation by plasmin

INVENTOR(S): Reed, Guy L.

PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA

SOURCE: PCT Int. Appl., 64 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9641883	A1	19961227	WO 1996-US9640	19960607
W: CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5854049	A	19981229	US 1995-488940	19950609
PRIORITY APPLN. INFO.:			US 1995-488940	A 19950609

AB **Streptokinase** analogs with altered plasmin-binding features that are resistant to binding by plasmin and subsequent cleavage and inactivation are described for use as thrombolytics with a prolonged serum half-life. Specifically, analogs of the *Streptococcus equisimilis* **streptokinase** are described. Changes that increase plasminogen resistance include alterations of the plasmin-binding domain and blocking of the N-terminus. Fusion proteins with maltose-binding protein as the N-terminal moiety are prepared and their plasmin resistance and **streptokinase** activity are described. Similarly, analogs with substitutions of basic amino acids that identify internal plasmin and trypsin cleavage sites were prepared and characterized.

L17 ANSWER 52 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1993:229181 HCAPLUS

DOCUMENT NUMBER: 118:229181

TITLE: **Cloning and expression** of a gene for **streptokinase** from a hemolytic *Streptococcus*

INVENTOR(S): Garcia, Mario P. E.; Chaplen, Roger R.; Hidalgo, Aimee P.; Doce, Ricardo S.; Marrero, Luciano F. H.; Collazo, Pedro R.; Ramirez, Anaisel C.; Munoz, Emilio A. M.; Martinez, Walfrido B.; et al.

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba

SOURCE: Can. Pat. Appl., 26 pp.

CODEN: CPXXEB

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2043953	AA	19921206	CA 1991-2043953	19910605
CA 2043953	C	20010612		



HU 62655	A2	19930528	HU 1991-1770	19910527
HU 216073	B	19990428		
SK 279873	B6	19990507	SK 1991-2256	19910719
PRIORITY APPLN. INFO.:			CA 1991-2043953	19910605

AB The gene for **streptokinase** of a *Streptococcus equisimilis* type C is cloned and expressed in *Escherichia coli* or in yeasts. Expression in yeasts uses the promoter of the AOX1 gene of *Pichia pastoris* to regulate expression. Secretion of the protein was achieved using the cognate signal peptide or one from sucrose invertase. The gene was cloned by PCR amplification using different pairs of primers to clone the gene with or without the signal sequence. Integrating expression vectors for expression of the gene in *Pichia pastoris* with or without secretion of the product were constructed. When the secretory construct was used, **streptokinase** yields of 1-1.2 g/L were obtained. The protein had the expected biol. activities, and the purified enzyme had a specific activity of 50,000-100,000 units/mg.

L17 ANSWER 53 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:646505 HCAPLUS

DOCUMENT NUMBER: 117:246505

TITLE: **Streptokinase** mutation affecting **skc** expression in homologous and heterologous hosts

AUTHOR(S): Mechold, U.; Muller, J.; Malke, H.

CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Jena, D-6900, Germany

SOURCE: Zentralblatt fuer Bakteriologie, Supplement (1992), 22(New Perspect. Streptococci Streptococcal Infect.), 336-8  
CODEN: ZBASE2; ISSN: 0941-018X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Mutations affecting the level of **streptokinase** gene **skc** expression and/or secretion in homologous and heterologous hosts are phys. characterized. The principal classes of mutations produced included **skc** deletions, IS element insertions, and **skc** duplications. The deletion events, represented by mutations  $\Delta(\text{skc})$ -247 and  $\Delta(\text{skc})$ -305 present in plasmids pMM247 and pMM305, resp., removed a tetrapeptide (F10-L13 or L12-A15) from the hydrophobic core of the Skc signal sequence. These mutations, reduced the size, hydrophobicity and predicted alpha-helicity of the central region of the signal sequence. The corresponding plasmids, upon transformation into *E. coli* and *P. mirabilis* L-forms, substantially increased the level of **Skc** expression in either host. In *E. coli*, they also facilitated the export of mature **Skc** into the culture medium. In the gram-pos. hosts, **skc** expression was less dramatically affected; however, the proportion of **Skc** activity found in the culture medium was significantly decreased when compared to the extracellular activity resulting from wild type **skc**. IS1 insertion did not alter the primary structure of the promoter but displaced in upward direction, by 768 bp, a static DNA bending locus having its center some 140 bp upstream of the -35 region in wild type DNA. When studied with plasmid pMM697, this insertion event resulted in severely decreased **Skc** expression in all hosts but, expectedly, did not affect **Skc** secretability. Gene **skc** duplication in the chromosome of the homologous producer strain, *S. equisimilis* H46A, was achieved by a single crossover event between the chromosomes and an integrateable **Skc** plasmid, pSM752, in the region of shared homol. As judged by Southern hybridization, cells transiently supporting the replication of pSM752 gave rise to a stable erythromycin-resistant clone designated H46SM which was plasmid-free and produced **Skc** at levels approx. twice as high as the wild type.

L17 ANSWER 54 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:585755 HCAPLUS

DOCUMENT NUMBER: 117:185755

TITLE: High-level **expression** of degraded product-free **streptokinase** in *Escherichia coli* by removal of its putative leader sequence. [Erratum to document cited in CA116(13):122160m]

AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung

CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea

SOURCE: Molecules and Cells (1992), 2(1), 119

CODEN: MOCEEK; ISSN: 1016-8478

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The omission of acknowledgment of a research grant has been corrected The error was not reflected in the abstract or the index entries.

L17 ANSWER 55 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:441935 HCAPLUS

DOCUMENT NUMBER: 117:41935

TITLE: Cloning and **expression** of **streptokinase** gene of C-type *Streptococcus equisimilis*

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba

SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04030794	A2	19920203	JP 1990-201600	19900731
JP 3127298	B2	20010122		
EP 489201	A1	19920610	EP 1990-201930	19900717
EP 489201	B1	19951115		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 130369	E	19951215	AT 1990-201930	19900717
ES 2081909	T3	19960316	ES 1990-201930	19900717
US 5296366	A	19940322	US 1991-703778	19910522
AU 644657	B2	19931216	AU 1991-78101	19910531
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A 19900523
			SU 1991-5001280	A 19910717

AB The **streptokinase** (I) gene **SKC-2**

,with/without signal sequence, is **cloned** from C-type *S.*

**equisimilis** ATCC-9542 by the polymerase chain reaction method and

**expressed** in *Escherichia coli* and yeast for com. manufacture

of I. Genomic DNA of the C-type *S. equisimilis* was isolated by

the standard method and amplified with primers derived from the nucleotide sequence of **SKC** to get I gene with/without signal sequence.

**Expression** of the I gene in *E. coli* and *Pichia pastoris*

MP-36 mutant were shown. The production of I with these microorganisms were  $\geq 350$  mg/L and  $\geq 1.2$  g/L, resp.

L17 ANSWER 56 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:122160 HCAPLUS

DOCUMENT NUMBER: 116:122160

TITLE: High-level **expression** of degraded

product-free **streptokinase** in *Escherichia coli* by removal of its putative leader sequence

AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung

CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea

SOURCE: Molecules and Cells (1991), 1(2), 187-92  
CODEN: MOCEEK; ISSN: 1016-8478

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **expression** vector for **streptokinase** has been constructed from the previously cloned **streptokinase** -coding gene (**skc**) from *Streptococcus equisimilis*. Because of its deleterious effect on the host cell growth, the leader sequence of **skc** was removed and the leader sequence-deleted **skc** was subcloned into the vector pKK223-3, which contains the regulatable tac promoter and rrnB T1T2 transcription terminator, with a short synthetic oligonucleotide adapter. When this vector, pKS601 having **skc** gene, was **expressed** in *E. coli*, a 47.4-kDa protein was found to be newly accumulated to about 12% of the total cellular proteins, and it was identified as the **streptokinase** by immunoblotting with rabbit anti-**streptokinase** polyclonal serum. The **expressed streptokinase** was free from carboxyl-terminal degraded 44-kDa **streptokinase** and purified to near homogeneity using DEAE-cellulose and Sephadex G-150 columns. Its specific activity was about 1.3 + 105 CLN units/mg protein.

L17 ANSWER 57 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:672660 HCAPLUS

DOCUMENT NUMBER: 115:272660

TITLE: **Recombinant** of thrombolytic and fibrinolytic enzymes as inactive dimers linked by sequence recognized by blood coagulation factors

INVENTOR(S): Dawson, Keith Martyn; Hunter, Michael George; Czaplewski, Lloyd George

PATENT ASSIGNEE(S): British Bio-Technology Ltd., UK

SOURCE: PCT Int. Appl., 110 pp.  
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 4

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9109125	A1	19910627	WO 1990-GB1911	19901207
W: AU, CA, FI, HU, JP, KR, NO, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
CA 2069085	AA	19910608	CA 1990-2069085	19901207
CA 2069085	C	20000201		
CA 2069105	AA	19910608	CA 1990-2069105	19901207
AU 9169540	A1	19910718	AU 1991-69540	19901207
AU 644399	B2	19931209		
ZA 9009853	A	19920826	ZA 1990-9853	19901207
ZA 9009854	A	19920826	ZA 1990-9854	19901207
EP 504241	A1	19920923	EP 1991-900869	19901207
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 155812	E	19970815	AT 1991-900851	19901207
ES 2106073	T3	19971101	ES 1991-900851	19901207
IL 96601	A1	19990509	IL 1990-96601	19901207
JP 05502374	T2	19930428	JP 1991-501314	19911115
JP 2900606	B2	19990602		
US 5434073	A	19950718	US 1992-854596	19920603

FI 9202609	A	19920605	FI 1992-2609	19920605
NO 9202237	A	19920806	NO 1992-2237	19920605
AU 9344976	A1	19931118	AU 1993-44976	19930830
PRIORITY APPLN. INFO.:			GB 1989-27722	A 19891207
			WO 1990-GB1911	A 19901207

AB Fibrinolytic or thrombolytic enzymes are manufactured in a **recombinant** host as inactive fusion proteins containing two or more sequences of the protein linked by a peptide that can be cleaved by a blood-coagulation factor. The construction of **expression** vectors for the manufacture of hirudin or **streptokinase** dimers linked by peptides cleavable by Factor Xa or thrombin for *Escherichia coli* or *Saccharomyces cerevisiae* (with or without product secretion) is described. All the products tested were cleavable by the appropriate factors.

L17 ANSWER 58 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:443480 HCAPLUS

DOCUMENT NUMBER: 115:43480

TITLE: Synthetic genes for **streptokinase** and **streptokinase** analogs and their **expression** in *Escherichia coli*

INVENTOR(S): Fujii, Setsuro; Katano, Tamiki; Majima, Eiji; Ogino, Koichi; Ono, Kenji; Sakata, Yasuyo; Uenoyama, Tsutomu

PATENT ASSIGNEE(S): Otsuka Pharmaceutical Factory, Inc., Japan

SOURCE: Eur. Pat. Appl., 76 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 407942	A2	19910116	EP 1990-113099	19900709
EP 407942	A3	19910904		
EP 407942	B1	19951011		
R: AT, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
JP 04011892	A2	19920116	JP 1990-179851	19900706
US 5240845	A	19930831	US 1990-549049	19900706
AU 9058806	A1	19910117	AU 1990-58806	19900709
AU 648029	B2	19940414		
AT 129014	E	19951015	AT 1990-113099	19900709
ES 2078925	T3	19960101	ES 1990-113099	19900709
CA 2020828	AA	19910112	CA 1990-2020828	19900710

PRIORITY APPLN. INFO.:

JP 1989-179432	A	19890711
JP 1989-307957	A	19891127
JP 1990-96830	A	19900411

AB Genes encoding **streptokinase** (I) and its derivs. are synthesized and **expressed** in a host such as *Escherichia coli* for manufacture of I suitable for clin. application. The DNA encoding natural-type I was synthesized by standard chemical and used for construction of **expression** plasmid pSKXT, which in turn **expressed** the I gene using the *E. coli* tac promoter and the blc signal sequence. Efficient **expression** of the gene in the *E. coli* transformants and purification of the protein product were demonstrated. I analogs with a carboxy-terminal deletions, optionally with internal modifications were also described.

L17 ANSWER 59 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:179754 HCAPLUS

DOCUMENT NUMBER: 114:179754

TITLE: Fusion proteins of **streptokinase** and human plasminogen

INVENTOR(S): Malke, Horst; Ferretti, Joseph J.

PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Zentralinstitut

fuer Mikrobiologie und Experimentelle Therapie, Ger.  
 Dem. Rep.  
 SOURCE: Ger. (East), 42 pp.  
 CODEN: GEXXA8  
 DOCUMENT TYPE: Patent  
 LANGUAGE: German  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 284484	A5	19901114	DD 1989-328631	19890516
PRIORITY APPLN. INFO.:			DD 1989-328631	19890516

AB A hybrid **streptokinase** is produced in prokaryotic cells. The **streptokinase**, which displays thrombin selectivity, consists of the N-terminal kringle domains of human plasminogen fused to C-terminal *Streptococcus equisimilis* **streptokinase**. *Escherichia coli* transformed with plasmids encoding the described fusion protein fused to the N-terminal hexapeptide of  $\beta$ -galactosidase produced the hybrid **streptokinase** which was purified from cell lysates by immuno-affinity chromatog. and by chromatog. on lysine Sepharose.

L17 ANSWER 60 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:162447 HCAPLUS  
 DOCUMENT NUMBER: 114:162447  
 TITLE: Recombinant manufacture of **streptokinase**  
 INVENTOR(S): Laplace, Frank; Mueller, Joerg; Malke, Horst  
 PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Patentabteilung, Ger. Dem. Rep.  
 SOURCE: Ger. (East), 9 pp.  
 CODEN: GEXXA8  
 DOCUMENT TYPE: Patent  
 LANGUAGE: German  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 282709	A5	19900919	DD 1988-323948	19881227
PRIORITY APPLN. INFO.:			DD 1988-323948	19881227

AB **Streptokinase** from *Streptococcus equisimilis* serotype C is manufactured by **expression** of the *skc* gene in *Escherichia coli*, *Bacillus subtilis*, or other *Streptococcus*. The natural **expression** cassette for the *skc* gene was introduced into a broad host-range vector to give plasmid pMLS10. Transformants of *Streptococcus sanguis* carrying this vector produced 750-1,000 **streptokinase** units/mL in a complex medium after 16 h growth at 36°, at this point the culture reached stationary phase and the enzyme continued to be slowly accumulated.

L17 ANSWER 61 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:96194 HCAPLUS  
 DOCUMENT NUMBER: 114:96194  
 TITLE: The leader sequence of **streptokinase** is responsible for its post-translational carboxyl-terminal cleavage  
 AUTHOR(S): Park, Seung Kook; Lee, Byeong Ryong; Byun, Si Myung  
 CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea  
 SOURCE: Biochemical and Biophysical Research Communications (1991), 174(1), 282-6  
 CODEN: BBRCA9; ISSN: 0006-291X

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB When the **streptokinase** gene from *Streptococcus equisimillis* was expressed from 2 tac promoter-controlled **expression** vectors, one deleted the putative leader sequence of **streptokinase**. Both normal and degraded **streptokinase** were detected in proteins expressed from the leader-encoding vector, but only normal **streptokinase** was detected from the leader-deleted vector. These findings indicate that the characteristic carboxyl-terminal cleavage of **streptokinase** is correlated with its leader sequence and occurs during defective secretion. A homogeneous preparation of **streptokinase** was facilitated by **expression** from this leader-deleted vector.

L17 ANSWER 62 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1989:206803 HCAPLUS

DOCUMENT NUMBER: 110:206803

TITLE: **Streptokinase** mutations relieving  
Escherichia coli K-12 (prlA4) of detriments  
caused by the wild-type **skc** gene

AUTHOR(S): Mueller, Joerg; Reinert, Hilmer; Malke, Horst  
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Therapy, Acad. Sci. G. D.  
R., Jena, 6900, Ger. Dem. Rep.

SOURCE: Journal of Bacteriology (1989), 171(4), 2202-8  
CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A novel phenotype is described for *E. coli* K-12 carrying the prlA4 allele determining a membrane component of the protein export mechanism. It is manifest as transformation deficiency for plasmids containing the cloned group C streptococcal **streptokinase** gene, **skc**. **Streptokinase** plasmid mutations relieving the prlA4 strain of this deficiency fell into three classes. Class 1 included **skc::IS5** insertions, with IS5 integrated in a region encoding the **Skc** signal sequence and inactivating **skc**. Class 2 included IS1 insertions leaving **skc** intact but reducing **skc expression**, presumably by altering the function of the **skc** promoter as judged by an insertion site close to the -35 region. Class 3 included **skc** deletions removing the entire signal sequence or a tetrapeptide from its hydrophobic core. The tetrapeptide deletion reduced the size, hydrophobicity, and predicted  $\alpha$ -helicity of the central region of the **Skc** signal sequence but facilitated the export of mature **Skc** in both the wild type and the prlA4 mutant. These findings indicate that the incompatibility between prlA4 and **skc** is related to deleterious effects of the **Skc** signal sequence. The tetrapeptide deletion may function by altering the conformation of the signal sequence so as to render interaction with both the PrlA wild-type protein and the PrlA4 mutant protein less detrimental to the export mechanism. These findings also provide an explanation for the difficulties encountered in cloning **streptokinase** genes in *E. coli* plasmids and maintaining their structural stability.

L17 ANSWER 63 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1988:487410 HCAPLUS

DOCUMENT NUMBER: 109:87410

TITLE: Methylo-trophic yeast as vehicles for heterologous gene  
**expression**

AUTHOR(S): Stroman, D. W.; Hagenson, M. J.

CORPORATE SOURCE: Phillips Res. Cent., Phillips Pet. Co., OK, USA

SOURCE: DECHEMA Monographien (1987), 105(Physiol. Genet.  
Modulation Prod. Form.), 141-6  
CODEN: DMDGAG; ISSN: 0070-315X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The methylotrophic yeast, *Pichia pastoris*, has been developed as a superior recombinant DNA (rDNA) production host. The key component in the development of this host was the cloning of the alc. oxidase gene and use of its promoter-regulatory region to control gene expression. Heterologous expression of several foreign genes in this yeast has been studied. The promoter-regulatory region from the alc. oxidase gene permits very high per cell levels of gene expression in an easily regulated manner. These high per cell levels of expression can be combined with high cell d. fermentation technol. to yield very high per L production of rDNA products. This is shown by the high levels of production of streptokinase in this yeast.

L17 ANSWER 64 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1987:472094 HCAPLUS

DOCUMENT NUMBER: 107:72094

TITLE: New cloning vectors for *Escherichia coli* and *Bacillus subtilis*

INVENTOR(S): Klessen, Christian; Malke, Horst

PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Ger. Dem. Rep.

SOURCE: Ger. (East), 7 pp.

CODEN: GEXXA8

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 231083	A1	19851218	DD 1984-264255	19840619
PRIORITY APPLN. INFO.:			DD 1984-264255	19840619

AB New cloning vectors for *Escherichia coli* and *Bacillus subtilis*, derived from the bifunctional plasmid pGR71 by insertion of a promoter-containing DNA fragment into the unique HindIII site of pGR71 upstream of the chloramphenicol acetyltransferase gene, are described. The new plasmids pSM1711, pSM7711, and pSM7712 contain a *Streptococcus* promoter from plasmids pMF1, pSM10, or pSM7.

L17 ANSWER 65 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:509637 HCAPLUS

DOCUMENT NUMBER: 105:109637

TITLE: The streptokinase gene

AUTHOR(S): Malke, H.; Ferretti, J. J.

CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Ger. Acad. Sci., Jena, DDR-6900, Ger. Dem. Rep.

SOURCE: *Folia Haematologica* (Leipzig) (1986), 113(1-2), 88-98

CODEN: FOHEAW; ISSN: 0323-4347

DOCUMENT TYPE: Journal

LANGUAGE: English

AB *Streptococcus equisimilis* Gene *skc* for streptokinase [9002-01-1] was cloned on the vector L47 and then subcloned into plasmids pACYC184 and pBR322 to form recombinant plasmids pMF2 (10.4 kb) and pMF5 (9.9 kb), resp., for expression in *Escherichia coli*. Plasmid pMF5 contained a 2568-base-pair (bp) insert that included the 1320-bp coding sequence for prestreptokinase. The prestreptokinase comprised 440 amino acid residues, including a 26-amino acid signal peptide. The insert also contained the *skc* upstream regions involved in the regulation of transcription and translation and a 15-bp repeat located 34 bp downstream of the *skc* translation stop signal, which very likely represents the rho-independent transcription terminator. The *skc* gene showed no extended regions homologous to the staphylokinase gene. Heterologous *skc* gene expression was also attained in *S. sanguis* after subcloning of the gene of pMF5 onto plasmid pSM7 to form the

bifunctional shuttle plasmid pSM752 (13.3 kb). Plasmid pSM752 was not only functional in *E. coli* and *S. sanguis*, but also in *Bacillus subtilis*. The **cloned streptokinase expressed** in *E. coli*, *S. sanguis*, or *B. subtilis* has the same specificity as that of the donor strain.

L17 ANSWER 66 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:473709 HCAPLUS

DOCUMENT NUMBER: 105:73709

TITLE: Cloning of streptococcal genes with Streptococcus-Escherichia coli shuttle vector pSA3

AUTHOR(S): Dao, M. L.; Ferretti, J. J.

CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA

SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 233-4. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK. CODEN: 55BSAN

DOCUMENT TYPE: Conference

LANGUAGE: English

AB A shuttle vector, the chimeric plasmid pSA3, which can replicate in both *E. coli* and *S. sanguis*, was constructed. Chromosomal DNA from *S. mutans* was ligated into this plasmid and cloned in *E. coli*. Of 472 clones tested, 43 clones **expressed** *S. mutans* surface antigens. A cloned *S. equisimilis* **streptokinase** [9002-01-1] gene was inserted into plasmid pSA3 and then used to transform *E. coli*, *S. sanguis*, and *S. mutans*, all of which **expressed** the **cloned streptokinase** gene.

L17 ANSWER 67 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:473707 HCAPLUS

DOCUMENT NUMBER: 105:73707

TITLE: Cloned streptokinase gene from Streptococcus equisimilis H46A

AUTHOR(S): Malke, H.; Ferretti, J. J.

CORPORATE SOURCE: Ger. Acad. Sci., Jena, Ger. Dem. Rep.

SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 221-2. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK. CODEN: 55BSAN

DOCUMENT TYPE: Conference

LANGUAGE: English

AB The **streptokinase** [9002-01-1] gene *skc* of *S. equisimilis* was cloned in *Escherichia coli* with plasmid pBR322. **Expression** of gene *skc* was observed with both orientations of the gene, which indicated that its own promoter was present and was functional in *E. coli*. **Streptokinase** was excreted by the *E. coli* host. The gene contained a 1320-base-pair open reading frame which encodes 440 amino acids, including a signal peptide of 26 amino acids.

L17 ANSWER 68 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:455636 HCAPLUS

DOCUMENT NUMBER: 105:55636

TITLE: The streptokinase gene: cloning, sequencing and **expression** in new hosts

AUTHOR(S): Malke, Horst

CORPORATE SOURCE: Zentralinst. Mikrobiol., Dtsch. Akad. Wiss., Jena,



SOURCE: Ger. Dem. Rep.  
Zeitschrift fuer Klinische Medizin (1985) (1986),  
41(7), 502-4  
CODEN: ZKMEEF; ISSN: 0233-1608  
DOCUMENT TYPE: Journal  
LANGUAGE: German

AB The streptokinase (I) [9002-01-1] gene (skc) of Streptococcus equisimilis H46A was cloned in Escherichia coli using vector  $\lambda$ L47. One of the recombinant clones was used to subclone skc in E. coli plasmid vectors. Plasmids pMF2 (10.4 kilobases, composed of pACYC184 plus a 6.4-kilobase EcoRI fragment) and pMF5 (6.9 kilobases, with a 2.5-kilobase fragment in the PstI site of pBR322) determined I formation in E. coli; expression of skc was independent of its orientation, indicating that the complete gene, together with its control elements, was present. The 2.5-kilobase PstI fragment of pMF5 was isolated and sequenced in the M13 system. Of 2568 base pairs, the largest open reading frame consisted of 1320 base pairs coding for prestreptokinase, corresponding to I plus its 26-amino acid leader sequence. Expression of skc was attained in S. sanguis after transformation with the shuttle vector pSM752. In fermentation expts., I production rates of 1500 U/mL were attained, which was below the levels obtained with S. equisimilis. Use of pSM752 for similar transformation of Bacillus subtilis is briefly discussed.

L17 ANSWER 69 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 1985:608113 HCAPLUS  
DOCUMENT NUMBER: 103:208113  
TITLE: Streptokinase-coding recombinant vectors  
INVENTOR(S): Ferretti, Joseph J.; Malke, Horst  
PATENT ASSIGNEE(S): Phillips Petroleum Co. , USA  
SOURCE: Eur. Pat. Appl., 21 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 151337	A2	19850814	EP 1984-306851	19841008
EP 151337	A3	19861008		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
DD 249037	A1	19870826	DD 1983-255523	19831010
US 4764469	A	19880816	US 1984-585417	19840302
AU 8433859	A1	19850418	AU 1984-33859	19841005
AU 561372	B2	19870507		
ZA 8407873	A	19850529	ZA 1984-7873	19841008
AT 61816	E	19910415	AT 1984-306851	19841008
FI 8403963	A	19850411	FI 1984-3963	19841009
NO 8404039	A	19850411	NO 1984-4039	19841009
DK 8404822	A	19850426	DK 1984-4822	19841009
JP 60237995	A2	19851126	JP 1984-212403	19841009
ES 536623	A1	19870116	ES 1984-536623	19841009
CA 1223223	A1	19870623	CA 1984-464939	19841009
DD 273284	A5	19891108	DD 1984-268254	19841010
US 5066589	A	19911119	US 1988-212254	19880627
US 5187098	A	19930216	US 1992-888420	19920522
PRIORITY APPLN. INFO.:			DD 1983-255523	A 19831010
			US 1984-585417	A 19840302
			EP 1984-306851	A 19841008
			US 1988-212254	A2 19880627
			US 1989-348206	B1 19890509

AB Recombinant vectors that code for streptokinase [9002-01-1] are constructed and cloned in *Escherichia coli*. Thus, DNA from *Streptococcus equisimilis* was isolated and digested with the restriction endonuclease *Sau3A*. DNA fragments of between 4-15 kb were cloned into phage  $\lambda$  L47. The ligated phage was infectively added to *E. coli* lawns and streptokinase-producing clones were isolated. The DNA from one such clone,  $\lambda$  L47E skc was partially digested with *HindIII* and then inserted into the *HindIII* site of plasmid pBR322. The recombinant plasmids were used to transform *E. coli* strain HB101. The plasmid isolated from 1 streptokinase-producing strain, pMF1, was isolated and a restriction map was prepared. A nucleotide sequence anal. of pMF1 showed that the cloned fragment encoded for streptokinase as well as an amino-terminal signal peptide which is bound to streptokinase and which is hydrolyzed during a streptokinase secretion event.

L17 ANSWER 70 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1985:107414 HCAPLUS

DOCUMENT NUMBER: 102:107414

TITLE: *Streptococcus-Escherichia coli* shuttle vector pSA3 and its use in the cloning of streptococcal genes

AUTHOR(S): Dao, My Lien; Ferretti, Joseph J.

CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, 73190, USA

SOURCE: Applied and Environmental Microbiology (1985), 49(1), 115-19

CODEN: AEMIDF; ISSN: 0099-2240

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A shuttle vector that can replicate in both *Streptococcus* and *E. coli* was constructed by joining the *E. coli* plasmid pACYC184 (chloramphenicol [56-75-7] and tetracycline [60-54-8] resistance) to the streptococcal plasmid pGB305 (erythromycin [114-07-8] resistance). The resulting chimeric plasmid is designated pSA3 (chloramphenicol, erythromycin, and tetracycline resistance) and had 7 unique restriction sites: *EcoRI*, *EcoRV*, *BamHI*, *SalI*, *XbaI*, *NruI*, and *SphI*. Mol. cloning into the *EcoRI* or *EcoRV* site results in inactivation of chloramphenicol resistance, and cloning into the *BamHI*, *SalI* site results in inactivation of tetracycline resistance in *E. coli*. Plasmid pSA3 was transformed and was stable in *S. sanguis* and *S. mutans* in the presence of erythromycin. Plasmid pSA3 was used to construct a library of the *S. mutans* GS5 genome in *E. coli*, and expression of surface antigens in this heterologous host was confirmed with *S. mutans* antiserum. A previously cloned determinant that species streptokinase [9002-01-1] was subcloned into pSA3, and this recombinant plasmid was stable in the presence of a selective pressure and expressed streptokinase activity in *E. coli*, *S. sanguis*, and *S. mutans*.

L17 ANSWER 71 OF 71 LIFESCI COPYRIGHT 2005 CSA on STN

ACCESSION NUMBER: 96:44932 LIFESCI

TITLE: Functional analysis of a *relA/spoT* gene homolog from *Streptococcus equisimilis*

AUTHOR: Mechold, U.; Cashel, M.; Steiner, K.; Gentry, D.; Malke, H.

CORPORATE SOURCE: Inst. Molecular Biol., Jena Univ., Winzerlaer Str. 10, D-07745 Jena, Germany

SOURCE: J. BACTERIOL., (1996) vol. 178, no. 5, pp. 1404-1411.

ISSN: 0021-9193.

DOCUMENT TYPE: Journal

FILE SEGMENT: J; G

LANGUAGE: English

SUMMARY LANGUAGE: English

AB We examined the functional attributes of a gene encountered by sequencing the streptokinase gene region of Streptococcus equisimilis H46A. This gene, originally called rel, here termed rel sub( )S. equisimilis, is homologous to two related Escherichia coli genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. coli mutants led us to deduce that the highly expressed rel sub( )S. equisimilis gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel sub( )S. equisimilis protein, purified from an E. coli relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel sub( )S. equisimilis protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel sub( )S. equisimilis protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel sub( )S. equisimilis gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel sub( )S. equisimilis in S. equisimilis abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

=> d his

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

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L1      42118 S STREPTOKINASE?
L2      630 S "SKC" OR "SKC-2"
L3      42539 S L1 OR L2
L4      7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5      4154 S L3 AND L4
L6      1362 S EQUISIMILIS
L7      220 S L5 AND L6
L8      49433 S INCLUSION (W) BOD?
L9      5 S L7 AND L8
L10     1 DUP REM L9 (4 DUPLICATES REMOVED)
L11     106 DUP REM L7 (114 DUPLICATES REMOVED)
L12     5 S LAMNDA
L13     1 S L11 AND INCLUSION
L14     0 S L11 AND AGGREGAT?
L15     0 S L11 AND INSOLUBLE
L16     1 S L11 AND SOLUBL?
L17     71 S L11 AND COLI
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=> e kuppusamy m/au

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E2      3      KUPPUSAMY KAVITHA T/AU
E3      40 --> KUPPUSAMY M/AU
E4      2      KUPPUSAMY M R/AU
E5      1      KUPPUSAMY MUSAVAN/AU
E6      2      KUPPUSAMY N/AU
E7      2      KUPPUSAMY NALLAGOUNDER/AU
E8      494     KUPPUSAMY P/AU
E9      1      KUPPUSAMY PARIANNAN/AU
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E10	1	KUPPUSAMY PERIANNAM/AU
E11	297	KUPPUSAMY PERIANNAN/AU
E12	1	KUPPUSAMY R/AU

=> s e3

L18 40 "KUPPUSAMY M"/AU

=> e ella k/au

E1	6	ELLA J/AU
E2	7	ELLA JUHA/AU
E3	0 -->	ELLA K/AU
E4	1	ELLA K A/AU
E5	53	ELLA K M/AU
E6	1	ELLA KAZACHKOVA/AU
E7	1	ELLA KHALID A ABOU/AU
E8	1	ELLA KIRSHNA M/AU
E9	1	ELLA KRISHNA E/AU
E10	28	ELLA KRISHNA M/AU
E11	2	ELLA KRISHNA MURTHY/AU
E12	1	ELLA KUSHNIR/AU

=> e khatri g s/au

E1	17	KHATRI G K/AU
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E3	46 -->	KHATRI G S/AU
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E7	15	KHATRI GHAN SHYAM/AU
E8	2	KHATRI GOPAL KRISHAN/AU
E9	1	KHATRI GS/AU
E10	1	KHATRI GULSHAN R/AU
E11	3	KHATRI H/AU
E12	1	KHATRI H K/AU

=> s e3

L19 46 "KHATRI G S"/AU

=> e lahiri s/au

E1	6	LAHIRI RANGAN/AU
E2	4	LAHIRI ROMA/AU
E3	1660 -->	LAHIRI S/AU
E4	2	LAHIRI S */AU
E5	8	LAHIRI S A/AU
E6	16	LAHIRI S B/AU
E7	610	LAHIRI S C/AU
E8	1	LAHIRI S C */AU
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E10	18	LAHIRI S D/AU
E11	1	LAHIRI S H/AU
E12	276	LAHIRI S K/AU

=> s e3

L20 1660 "LAHIRI S"/AU

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E1	656	SRINIVAS V/AU
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E3	28 -->	SRINIVAS V K/AU
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E5	89	SRINIVAS V R/AU
E6	43	SRINIVAS V S/AU
E7	1	SRINIVAS V SEENA/AU
E8	1	SRINIVAS V SESA/AU

E9 1 SRINIVAS V T/AU  
E10 7 SRINIVAS V V/AU  
E11 2 SRINIVAS VANKEEPURAM S/AU  
E12 2 SRINIVAS VELLIMEDU KANNAPPA/AU

=> s e3

L21 28 "SRINIVAS V K"/AU

=> d his

(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

L1 42118 S STREPTOKINASE?  
L2 630 S "SKC" OR "SKC-2"  
L3 42539 S L1 OR L2  
L4 7454183 S CLON? OR EXPRESS? OR RECOMBINANT  
L5 4154 S L3 AND L4  
L6 1362 S EQUISIMILIS  
L7 220 S L5 AND L6  
L8 49433 S INCLUSION (W) BOD?  
L9 5 S L7 AND L8  
L10 1 DUP REM L9 (4 DUPLICATES REMOVED)  
L11 106 DUP REM L7 (114 DUPLICATES REMOVED)  
L12 5 S LAMNDA  
L13 1 S L11 AND INCLUSION  
L14 0 S L11 AND AGGREGAT?  
L15 0 S L11 AND INSOLUBLE  
L16 1 S L11 AND SOLUBL?  
L17 71 S L11 AND COLI  
E KUPPUSAMY M/AU  
L18 40 S E3  
E ELLA K/AU  
E KHATRI G S/AU  
L19 46 S E3  
E LAHIRI S/AU  
L20 1660 S E3  
E SRINIVAS V K/AU  
L21 28 S E3

=> s l17 or l18 or l19 or l20 or l21

L22 1842 L17 OR L18 OR L19 OR L20 OR L21

=> s l3 and l22

L23 71 L3 AND L22

=> s l8 and l23

L24 1 L8 AND L23

=> d all

L24 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
AN 2000:96119 BIOSIS  
DN PREV200000096119  
TI Two **streptokinase** genes are **expressed** with different  
solubility in *Escherichia coli* W3110.  
AU Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria;  
Fernandez, Julio; Paez, Rolando; Torrens, Isis  
CS Biopharmaceutical Development Division, Center for Genetic Engineering and  
Biotechnology, Havana, Cuba  
SO Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.  
CODEN: BILED3. ISSN: 0141-5492.  
DT Article

LA English  
 ED Entered STN: 15 Mar 2000  
 Last Updated on STN: 3 Jan 2002  
 AB The **streptokinase** (SK) gene from *S. equisimilis* H46A (ATCC 12449) was cloned in *E. coli* W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from *S. equisimilis* (ATCC 9542) which had been cloned in *E. coli* W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of **inclusion bodies**.  
 CC Genetics of bacteria and viruses 31500  
 Biochemistry methods - Nucleic acids, purines and pyrimidines 10052  
 Biochemistry methods - Proteins, peptides and amino acids 10054  
 Replication, transcription, translation 10300  
 Biophysics - Molecular properties and macromolecules 10506  
 Microbiological apparatus, methods and media 32000  
 Food microbiology - General and miscellaneous 39008  
 Enzymes - General and comparative studies: coenzymes 10802  
 Metabolism - Proteins, peptides and amino acids 13012  
 Morphology and cytology of bacteria 30500  
 Physiology and biochemistry of bacteria 31000  
 IT Major Concepts  
 Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics (Biochemistry and Molecular Biophysics)  
 IT Chemicals & Biochemicals  
 amino acids; enzymes; proteins; tryptophan  
 IT Miscellaneous Descriptors  
 biotechnology; cell growth conditions; **expression** vectors; gene **expression**; promoters; tryptophan promoter  
 ORGN Classifier  
 Enterobacteriaceae 06702  
 Super Taxa  
 Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms  
 Organism Name  
*Escherichia coli*: W 3110  
 Taxa Notes  
 Bacteria, Eubacteria, Microorganisms  
 ORGN Classifier  
 Gram-Positive Cocci 07700  
 Super Taxa  
 Eubacteria; Bacteria; Microorganisms  
 Organism Name  
*Streptococcus equisimilis*  
 Taxa Notes  
 Bacteria, Eubacteria, Microorganisms  
 RN 54-12-6Q (tryptophan)  
 73-22-3Q (tryptophan)

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

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 L16 1 S L11 AND SOLUBL?  
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 E KUPPUSAMY M/AU  
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 E ELLA K/AU  
 E KHATRI G S/AU  
 L19 46 S E3  
 E LAHIRI S/AU  
 L20 1660 S E3  
 E SRINIVAS V K/AU  
 L21 28 S E3  
 L22 1842 S L17 OR L18 OR L19 OR L20 OR L21  
 L23 71 S L3 AND L22  
 L24 1 S L8 AND L23

=> s l4 and l23  
 L25 71 L4 AND L23

=> s l6 and l25  
 L26 71 L6 AND L25

=> d his

(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,  
 LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

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 L7 220 S L5 AND L6  
 L8 49433 S INCLUSION (W) BOD?  
 L9 5 S L7 AND L8  
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 L14 0 S L11 AND AGGREGAT?  
 L15 0 S L11 AND INSOLUBLE  
 L16 1 S L11 AND SOLUBL?  
 L17 71 S L11 AND COLI  
 E KUPPUSAMY M/AU  
 L18 40 S E3  
 E ELLA K/AU  
 E KHATRI G S/AU  
 L19 46 S E3  
 E LAHIRI S/AU  
 L20 1660 S E3  
 E SRINIVAS V K/AU  
 L21 28 S E3  
 L22 1842 S L17 OR L18 OR L19 OR L20 OR L21  
 L23 71 S L3 AND L22

L24 1 S L8 AND L23  
L25 71 S L4 AND L23  
L26 71 S L6 AND L25

=> d 1-71 ibib ab

L26 ANSWER 1 OF 71 MEDLINE on STN  
ACCESSION NUMBER: 2002322701 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12065504  
TITLE: Dual control of **streptokinase** and streptolysin S  
production by the covRS and fasCAX two-component regulators  
in *Streptococcus dysgalactiae* subsp. **equisimilis**.  
AUTHOR: Steiner Kerstin; Malke Horst  
CORPORATE SOURCE: Institute for Molecular Biology, Friedrich Schiller  
University Jena, D-07745 Jena, Germany.  
SOURCE: Infection and immunity, (2002 Jul) 70 (7) 3627-36.  
Journal code: 0246127. ISSN: 0019-9567.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AY075106; GENBANK-AY075107  
ENTRY MONTH: 200207  
ENTRY DATE: Entered STN: 20020615  
Last Updated on STN: 20020731  
Entered Medline: 20020730

AB Synthesis of the plasminogen activator **streptokinase** (SK) by group A streptococci (GAS) has recently been shown to be subject to control by two two-component regulators, covRS (or csrRS) and fasBCA. In independent studies, response regulator CovR proved to act as the repressor, whereas FasA was found to act indirectly as the activator by controlling the **expression** of a stimulatory RNA, fasX. In an attempt at understanding the regulation of SK production in the human group C streptococcal (GCS) strain H46A, the strongest SK producer known yet, we provide here physical and functional evidence for the presence of the cov and fas systems in GCS as well and, using a mutational approach, compare the balance between their opposing actions in H46A and GAS strain NZ131. Sequence analysis combined with Southern hybridization revealed that the covRS and fasCAX operons are preserved at high levels of primary structure identity between the corresponding GAS and GCS genes, with the exception of fasB, encoding a second sensor kinase that is not a member of the GCS fas operon. This analysis also showed that wild-type H46A is actually a derepressed mutant for SK and streptolysin S (SLS) synthesis, carrying a K102 amber mutation in covR. Using cov and fas mutations in various combinations together with strain constructs allowing complementation in trans, we found that, in H46A, cov and fas contribute to approximately equal negative and positive extents, respectively, to constitutive SK and SLS activity. The amounts of SK paralleled the level of skc(H46A) transcription. The most profound difference between H46A and NZ131 regarding the relative activities of the cov and fas systems consisted in significantly higher activity of a functional CovR repressor in NZ131 than in H46A. In NZ131, CovR decreased SK activity in a Fas(+) background about sevenfold, compared to a 1.9-fold reduction of SK activity in H46A. Combined with the very short-lived nature of covR mRNA (decay rate, 1.39/min), such differences may contribute to strain-specific peculiarities of the **expression** of two prominent streptococcal virulence factors in response to environmental changes.

L26 ANSWER 2 OF 71 MEDLINE on STN  
ACCESSION NUMBER: 2002053807 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 11779212  
TITLE: Specificity role of the **streptokinase** C-terminal domain in plasminogen activation.



AUTHOR: Kim Dong Min; Lee Sang Jun; Yoon Suk Kwon; Byun Si Myung  
 CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 305-701 Taejeon, South Korea.  
 SOURCE: Biochemical and biophysical research communications, (2002 Jan 11) 290 (1) 585-8.  
 Journal code: 0372516. ISSN: 0006-291X.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200202  
 ENTRY DATE: Entered STN: 20020125  
 Last Updated on STN: 20020226  
 Entered Medline: 20020225

AB Several pathogenic bacteria secrete plasminogen activator proteins. **Streptokinase** (SKe) produced by *Streptococcus equisimilis* and staphylokinase secreted from *Staphylococcus aureus* are human plasminogen activators and **streptokinase** (SKu), produced by *Streptococcus uberis*, is a bovine plasminogen activator. Thus, the fusion proteins among these activators can explain the function of each domain of SKe. Replacement of the SKalpha domain with staphylokinase donated the staphylokinase-like activation activity to SKe, and the SKbetagamma domain played a role of nonproteolytic activation of plasminogen. **Recombinant** SKu also activated human plasminogen by staphylokinase-like activation mode. Because SKu has homology with SKe, the bovine plasminogen activation activities of SKe fragments were checked. SKebetagamma among them had activation activity with bovine plasminogen. This means that the C-terminal domain (gamma-domain) of **streptokinase** determines plasminogen species necessary for activation and converses the ability of substrate recognition to human species.  
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L26 ANSWER 3 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 2000038313 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 10569766  
 TITLE:

**Cloning, expression, sequence analysis, and characterization of streptokinases** secreted by porcine and equine isolates of *Streptococcus equisimilis*.

AUTHOR: Caballero A R; Lottenberg R; Johnston K H  
 CORPORATE SOURCE: Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans, Louisiana 70112, USA.

CONTRACT NUMBER: R01DK45014 (NIDDK)  
 SOURCE: Infection and immunity, (1999 Dec) 67 (12) 6478-86.  
 Journal code: 0246127. ISSN: 0019-9567.

PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-AF104300; GENBANK-AF104301  
 ENTRY MONTH: 199912  
 ENTRY DATE: Entered STN: 20000113  
 Last Updated on STN: 20000113  
 Entered Medline: 19991220

AB **Streptokinases** secreted by nonhuman isolates of group C streptococci (*Streptococcus equi*, *S. equisimilis*, and *S. zooepidemicus*) have been shown to bind to different mammalian plasminogens but exhibit preferential plasminogen activity. The **streptokinase** genes from *S. equisimilis* strains which activated either equine or porcine plasminogen were **cloned**, sequenced, and **expressed** in *Escherichia coli*. The

streptokinase secreted by the equine isolate had little similarity to any known streptokinases secreted by either human or porcine isolates. The streptokinase secreted by the porcine isolate had limited structural and functional similarities to streptokinases secreted by human isolates. Plasminogen activation studies with immobilized (His) (6)-tagged recombinant streptokinases indicated that these recombinant streptokinases interacted with plasminogen in a manner similar to that observed when streptokinase and plasminogen interact in the fluid phase. Analysis of the cleavage products of the streptokinase-plasminogen interaction indicated that human, equine, and porcine plasminogens were all cleaved at the same highly conserved site. The site at which streptokinase was cleaved to form altered streptokinase (Sk\*) was also determined. This study confirmed not only the presence of streptokinases in nonhuman *S. equisimilis* isolates but also that these proteins belong to a family of plasminogen activators more diverse than previously thought.

L26 ANSWER 4 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 1998350778 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 9686161  
 TITLE: Cloning, expression and purification of recombinant streptokinase: partial characterization of the protein expressed in *Escherichia coli*.  
 AUTHOR: Avilan L; Yarzabal A; Jurgensen C; Bastidas M; Cruz J; Puig J  
 CORPORATE SOURCE: Laboratorio de Biología y Medicina Experimental, Facultad de Ciencias, Universidad de Los Andes, Mérida, Venezuela.  
 SOURCE: Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.], (1997 Dec) 30 (12) 1427-30.  
 Journal code: 8112917. ISSN: 0100-879X.  
 PUB. COUNTRY: Brazil  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199810  
 ENTRY DATE: Entered STN: 19981020  
 Last Updated on STN: 19981020  
 Entered Medline: 19981005

AB We cloned the streptokinase (STK) gene of *Streptococcus equisimilis* in an expression vector of *Escherichia coli* to overexpress the profibrinolytic protein under the control of a tac promoter. Almost all the recombinant STK was exported to the periplasmic space and recovered after gentle lysozyme digestion of induced cells. The periplasmic fraction was chromatographed on DEAE Sepharose followed by chromatography on phenyl-agarose. Active proteins eluted between 4.5 and 0% ammonium sulfate, when a linear gradient was applied. Three major STK derivatives of 47.5 kDa, 45 kDa and 32 kDa were detected by Western blot analysis with a polyclonal antibody. The 32-kDa protein formed a complex with human plasminogen but did not exhibit Glu-plasminogen activator activity, as revealed by a zymographic assay, whereas the 45-kDa protein showed a  $K(m) = 0.70 \text{ microM}$  and  $k_{cat} = 0.82 \text{ s}^{-1}$ , when assayed with a chromogen-coupled substrate. These results suggest that these proteins are putative fragments of STK, possibly derived from partial degradation during the export pathway or the purification steps. The 47.5-kDa band corresponded to the native STK, as revealed by peptide sequencing.

L26 ANSWER 5 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 96397500 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8804394

TITLE: Cloning of heterologous genes specifying detrimental proteins on pUC-derived plasmids in *Escherichia coli*.

AUTHOR: Muller J; van Dijl J M; Venema G; Bron S

CORPORATE SOURCE: Institut fur Molekularbiologie, Friedrich-Schiller-Universitat Jena, Germany.

SOURCE: Molecular & general genetics : MGG, (1996 Aug 27) 252 (1-2) 207-11.  
Journal code: 0125036. ISSN: 0026-8925.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199610

ENTRY DATE: Entered STN: 19961219  
Last Updated on STN: 19961219  
Entered Medline: 19961031

AB A system is described that enables the cloning of genes specifying detrimental proteins in *Escherichia coli*. The system is based on pUC plasmids and was developed for the expression of the *Bacillus subtilis* *csaA* gene, which is lethal when expressed at high levels. Suppressor strains that tolerate the presence of plasmids for high-level expression of *csaA* were isolated, which contained small cryptic deletion variants of the parental plasmid in high copy numbers. The cryptic plasmids consisted mainly of the pUC replication functions and lacked the *csaA* region and selectable markers. The co-resident, incompatible, cryptic plasmids enabled the maintenance of the *csaA* plasmids by reducing their copy number 20-fold, which resulted in a concomitant 3- to 7-fold reduction in the expression of plasmid-encoded genes. Strains carrying these cryptic endogenous plasmids proved to be useful for the construction of pUC-based recombinant plasmids carrying other genes, such as the *skc* gene of *Streptococcus equisimilis*, which cannot be cloned in high copy numbers in *E. coli*. Several strategies to reduce production levels of heterologous proteins specified by plasmids are compared.

L26 ANSWER 6 OF 71 MEDLINE on STN

ACCESSION NUMBER: 96396845 MEDLINE

DOCUMENT NUMBER: PubMed ID: 8803948

TITLE: Structural dissection and functional analysis of the complex promoter of the streptokinase gene from *Streptococcus equisimilis* H46A.

AUTHOR: Grafe S; Ellinger T; Malke H

CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.

SOURCE: Medical microbiology and immunology, (1996 May) 185 (1) 11-7.  
Journal code: 0314524. ISSN: 0300-8584.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199701

ENTRY DATE: Entered STN: 19970219  
Last Updated on STN: 19970219  
Entered Medline: 19970131

AB The overlapping tandem promoters of the streptokinase gene, P1 and P2, identified previously by S1 nuclease transcript mapping were functionally dissected by mutagenesis of their -10 regions and fused transcriptionally with or without the 202-bp upstream region (USR) to the luciferase reporter gene (*luc*) from *Photinus pyralis* to analyze the contribution of the different sequence elements to promoter activity in *Escherichia coli* and the homologous *Streptococcus equisimilis* strain H46A. In *E. coli*, virtually the

entire promoter activity derived from the upstream promoter P1. In *S. equisimilis*, luc expression increased in the following order of the involved sequence elements: P2 approximately equal to P2 + USR < P1 < P1 + P2 < P1 + USR < P1 + P2 + USR. This shows that (1) in the homologous system, P1 and P2 alone are extremely weak, (2) in the USR-less arrangement, only the combined core promoters have substantial activity, and (3) the USR stimulates only P1 and the combination of P1 + P2. Thus, the tandem promoters presumably function by mutual contributory action and their full activity strongly depends on the AT-rich and statically bent upstream region. The distinctive feature determining the strength of P1 in both hosts appears to be its extended -10 region which matches the consensus TRTGN established for strong *S. pneumoniae* and *Bacillus subtilis* promoters.

L26 ANSWER 7 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 96305364 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8706717  
 TITLE: Cloning, sequencing and functional overexpression of the *Streptococcus equisimilis* H46A gapC gene encoding a glyceraldehyde-3-phosphate dehydrogenase that also functions as a plasmin(ogen)-binding protein. Purification and biochemical characterization of the protein.  
 AUTHOR: Gase K; Gase A; Schirmer H; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: European journal of biochemistry / FEBS, (1996 Jul 1) 239 (1) 42-51.  
 JOURNAL code: 0107600. ISSN: 0014-2956.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-X97788  
 ENTRY MONTH: 199609  
 ENTRY DATE: Entered STN: 19960919  
 Last Updated on STN: 19990129  
 Entered Medline: 19960910

AB We previously identified DNA sequences involved in the function of the complex promoter of the *streptokinase* gene from *Streptococcus equisimilis* H46A, a human serogroup C strain known to express this gene at a high level. As a prerequisite to understanding possible mechanisms that control the balance between the plasminogen activating and plasmin(ogen) binding capacities of H46A, we describe here its gapC gene encoding glyceraldehyde-3-phosphate dehydrogenase (GraP-DH, EC 1.2.1.12), a glycolytic enzyme apparently transported to the cell surface where it functions as a plasmin(ogen).binding protein. The gapC gene was cloned and sequenced and found to code for a 336-amino-acid polypeptide (approximately 35.9 kDa) exhibiting 94.9% sequence identity to the Plr protein from *Streptococcus pyogenes* shown by others to be capable of plasmin binding [Lottenberg, R., Broder, C. C., Boyle, M. D., Kain, S. J., Schroeder, B. L. & Curtiss, R. III (1992) J. Bacteriol. 174, 5204-5210]. To study the properties of the GapC protein, its gene was inducibly overexpressed in *Escherichia coli* from QIAexpress expression plasmids to yield the authentic GapC or (His)<sub>6</sub>GapC carrying a hexahistidyl N-terminus to permit affinity purification. Both proteins were functionally active, exhibiting specific GraP-DH activities of about 80 kat/mol (approximately 130 U/mg) after purification. Their binding parameters [association (k<sub>a</sub>) and dissociation (k<sub>d</sub>) rate constants, and equilibrium dissociation constants (K<sub>d</sub> = k<sub>d</sub>/k<sub>a</sub>)] for the interaction with human Gluplasminogen and plasmin were determined by real-time biospecific interaction analysis using the Pharmacia BIAcore instrument. For comparative purposes, the commercial GraP-DH from *Bacillus stearothermophilus* (BstGraP-DH), a nonpathogenic organism, was included in

these experiments. The Kd values for binding of plasminogen to GapC, (His)6GapC and BstGraP-DH were 220 nM, 260 nM and 520 nM, respectively, as compared to 25 nM, 17 nM and 98 nM, respectively, for the binding to plasmin. These data show that both the zymogen and active enzyme possess low-affinity binding sites for the gapC gene product and that the hexahistidyl terminus does not affect its function. Prior limited treatment with plasmin enhanced the subsequent plasminogen binding capacity of all three GraP-DHs, presumably by the exposure of new C-terminal lysine residues for binding to the zymogen.

L26 ANSWER 8 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 96200111 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8631718  
 TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus equisimilis.  
 AUTHOR: Mechold U; Cashel M; Steiner K; Gentry D; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Journal of bacteriology, (1996 Mar) 178 (5) 1401-11.  
 Journal code: 2985120R. ISSN: 0021-9193.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199607  
 ENTRY DATE: Entered STN: 19960715  
 Last Updated on STN: 19970203  
 Entered Medline: 19960703

AB We examined the functional attributes of a gene encountered by sequencing the streptokinase gene region of Streptococcus equisimilis H46A. This gene, originally called rel, here termed relS. equisimilis, is homologous to two related Escherichia coli genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. coli mutants led us to deduce that the highly expressed rel S. equisimilis gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel S. equisimilis protein, purified from an E. coli relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel S. equisimilis protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel S. equisimilis protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel S. equisimilis gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel S. equisimilis in S. equisimilis abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L26 ANSWER 9 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 96001243 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 7557478  
 TITLE: Secretion of streptokinase fusion proteins from Escherichia coli cells through the hemolysin transporter.  
 AUTHOR: Kern I; Ceglowski P  
 CORPORATE SOURCE: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa.  
 SOURCE: Gene, (1995 Sep 22) 163 (1) 53-7.  
 Journal code: 7706761. ISSN: 0378-1119.

PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199511  
ENTRY DATE: Entered STN: 19951227  
Last Updated on STN: 20021008  
Entered Medline: 19951108

AB The hemolysin (HlyA) secretion system was used to achieve the sec-independent secretion of **streptokinase (Skc)** originating from *Streptococcus equisimilis* into the medium by *Escherichia coli* cells. The in-frame fusions of the **skc** gene, either possessing or lacking a region encoding the signal peptide (SP) with the 3'-end of the hlyA gene of various lengths were analysed. All hybrids retained **Skc** activity. Hybrid proteins devoided of the N-terminal SP, regardless of length of the hlyA secretion signal (62 vs. 194 amino acids), were secreted into the medium by the *E. coli* HlyA transporter at similar levels. Considerable amounts of hybrid proteins were still, however, associated with *E. coli* cells, mainly in the degraded form.

L26 ANSWER 10 OF 71 MEDLINE on STN  
ACCESSION NUMBER: 95342169 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 7616967  
TITLE: Complex transcriptional control of the **streptokinase** gene of *Streptococcus equisimilis* H46A.  
AUTHOR: Gase K; Ellinger T; Malke H  
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
SOURCE: Molecular & general genetics : MGG, (1995 Jun 25) 247 (6) 749-58.  
Journal code: 0125036. ISSN: 0026-8925.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199508  
ENTRY DATE: Entered STN: 19950905  
Last Updated on STN: 19950905  
Entered Medline: 19950822

AB On the *Streptococcus equisimilis* H46A chromosome, the divergent coding sequences of the genes for the plasminogen activator **streptokinase (skc)** and a leucine-rich protein (lrp), the function of which is unknown, are separated by a 328 bp intrinsically bent DNA region rich in AT tracts. To begin to understand the **expression** control of these two genes, we mapped their transcriptional initiation sites by S1 nuclease analysis and studied the influence of the bent intergenic region on promoter strength, using promoter-reporter gene fusions of **skc'** and **lrp'** to 'lacZ from *Escherichia coli*. The major transcriptional start sites, in both *S. equisimilis* and *E. coli*, mapped 22 bases upstream of the ATG start site of lrp (G), and 24 and 32 bases upstream of the translational initiation codon of **skc** (A and G, respectively), indicating the existence of two overlapping canonical **skc** promoters arranged in tandem on opposite faces of the helix. The reporter gene fusions were cloned in *E. coli* on a vector containing a 1.1 kb fragment of the *S. equisimilis* dexB gene, thus allowing promoter strength to be measured in multiple plasmid-form copies in the heterologous host and in single-copy genomic form following integration into the **skc** region of the homologous host. In *S. equisimilis*, **skc'**-**'lacZ** was **expressed** about 200-fold more strongly than the corresponding **lrp'**-**'lacZ** fusion. In contrast, in *E. coli*, the corresponding levels of **expression** differed by only about 11-fold. Deletion

of the 202 bp bent region upstream of the *skc* and *lrp* core promoters caused a 13-fold decrease in *skc* promoter activity in *S. equisimilis* but did not alter *lrp* promoter strength in this host. In contrast, when studied in *E. coli*, this deletion did not alter the strength of the *skc*-double promoter and even increased by 2.4- to 3-fold the activity of the *lrp* promoter. This comparative promoter analysis shows that *skc* has a complex promoter structure, the activity of which in the homologous genomic environment specifically depends on sequences upstream of the two core promoters. Thus, the *skc* promoter structure resembles that of an array of promoters involved in a transcriptional switch; however, the nature of the potential switch factor(s) remains unknown.

L26 ANSWER 11 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 95157528 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 7531815  
 TITLE: Transcription termination of the *streptokinase* gene of *Streptococcus equisimilis* H46A: bidirectionality and efficiency in homologous and heterologous hosts.  
 AUTHOR: Steiner K; Malke H  
 CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.  
 SOURCE: Molecular & general genetics : MGG, (1995 Feb 6) 246 (3) 374-80.  
 Journal code: 0125036. ISSN: 0026-8925.  
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199503  
 ENTRY DATE: Entered STN: 19950322  
 Last Updated on STN: 19960129  
 Entered Medline: 19950316

AB In *Streptococcus equisimilis* H46A, a hypersymmetrical transcription terminator with bidirectional activity was localized between the translational termination codons of the *streptokinase* gene, *skc*, and the *rel-orf1* genes. These two transcription units are oriented towards each other, and under normal conditions the *skc* mRNA level exceeds that of the *rel-orf1* genes by a factor of at least 1000. Reporter vectors based on the promoterless *cat* gene were constructed by transcriptional fusion of *skc* to *cat*, such that the region between the two genes contained the terminator in *skc* orientation or in *rel-orf1* orientation. Additionally, *skc* and *cat* were fused directly, with deletion of the terminator. The reporter vectors were designed to be capable of being studied either as multicopy plasmids in *Escherichia coli* or in single copy following integration, via *skc*, into the *S. equisimilis* chromosome. Chloramphenicol acetyl transferase (CAT) activity assays in conjunction with determination of chloramphenicol resistance levels and Northern hybridization analysis showed that the terminator is active in either host and orientation. However, termination efficiency was host dependent, with high terminator strength being observed in the homologous streptococcal background and appreciable readthrough occurring in *E. coli*. The extent of transcriptional readthrough was dependent upon terminator orientation, with termination being more efficient in *rel-orf1* polarity. The results suggest that, in *S. equisimilis*, transcription of both *skc* and *rel-orf1* is efficiently terminated by a common signal, and that these genes are largely protected from convergent transcription, which otherwise would seem to be particularly detrimental to the weakly expressed *rel-orf1* genes.

L26 ANSWER 12 OF 71 MEDLINE on STN  
 ACCESSION NUMBER: 94049672 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8232196

TITLE: Genetic organization of the **streptokinase** region of the *Streptococcus equisimilis* H46A chromosome.

AUTHOR: Mechold U; Steiner K; Vettermann S; Malke H

CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.

SOURCE: Molecular & general genetics : MGG, (1993 Oct) 241 (1-2) 129-40.  
Journal code: 0125036. ISSN: 0026-8925.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-X72832

ENTRY MONTH: 199312

ENTRY DATE: Entered STN: 19940117  
Last Updated on STN: 19940117  
Entered Medline: 19931215

AB The complete nucleotide sequences of four genes and one open reading frame (ORF1) adjacent to the **streptokinase** gene, **skc**, from *Streptococcus equisimilis* H46A were determined. These genes are encoded on the opposite DNA strand to **skc** and are arranged as follows: **dexB-abc-lrp-skc-ORF1-rel**. The **dexB** gene, coding for an alpha-glucosidase (M(r) 61,733), and **abc**, encoding an ABC transporter (M(r) 42,080), are similar to the **dexB** and **msmK** genes, respectively, from the multiple sugar metabolism operon of *S. mutans*. The **lrp** gene specifies a leucine-rich protein (M(r) 32,302) that has a leucine-zipper motif at its C-terminus. The function of the Lrp protein is not known but appeared to be detrimental when overexpressed in *Escherichia coli*. Although **lrp** appears not to be an essential gene, as judged by plasmid insertion mutagenesis, it is conserved in all streptococcal strains carrying a **streptokinase** gene. The **rel** gene showed significant homology to the *E. coli* **relA** and **spoT** genes involved in the stringent response to amino acid deprivation. Multiple alignment of the amino acid sequences of **Rel** (M(r) 83,913), **RelA** and **SpoT** revealed 59.4% homology of the primary structures. Northern hybridization analyses of the genes in the **skc** region showed **skc** to be transcribed most abundantly. In addition to transcripts for **skc**, monocistronic mRNAs were detected for all three genes divergently transcribed from **skc**. Although there was also some read-through transcription from **lrp** into **abc**, and from **abc** into **dexB**, the transcription pattern suggests a high degree of transcriptional and functional independence not only of **skc** but also **abc** and **dexB**. Prominent structural features in intergenic regions included a static DNA bending locus located upstream and a putative bidirectional transcription terminator downstream of **skc**.

L26 ANSWER 13 OF 71 MEDLINE on STN

ACCESSION NUMBER: 92039051 MEDLINE

DOCUMENT NUMBER: PubMed ID: 1937032

TITLE: Isolation, sequence and **expression** in *Escherichia coli*, *Bacillus subtilis* and *Lactococcus lactis* of the DNase (streptodornase)-encoding gene from *Streptococcus equisimilis* H46A.

AUTHOR: Wolinowska R; Ceglowski P; Kok J; Venema G

CORPORATE SOURCE: Department of Pharmaceutical Microbiology, Medical Academy, Warsaw, Poland.

SOURCE: Gene, (1991 Sep 30) 106 (1) 115-9.  
Journal code: 7706761. ISSN: 0378-1119.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-M59725; GENBANK-M59726; GENBANK-M59727;  
GENBANK-M59728; GENBANK-M63990; GENBANK-S61507;  
GENBANK-S63856; GENBANK-S63863; GENBANK-S65020;



GENBANK-S65060; GENBANK-X17241

ENTRY MONTH: 199112

ENTRY DATE: Entered STN: 19920124

Last Updated on STN: 19920124

Entered Medline: 19911223

AB A partial library of BclI-generated chromosomal DNA fragments from *Streptococcus equisimilis* H64A (Lancefield Group C) was constructed in *Escherichia coli*. Clones displaying either **streptokinase** or deoxyribonuclease (**streptodornase**; SDC) activities were isolated. The gene (**sd**c) **expressing** the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The **sd**c gene was **expressed** in *E. coli*, *Bacillus subtilis* and *Lactococcus lactis*. As observed for *S. equisimilis*, in the heterologous Gram + hosts, at least part of the SDC protein was secreted into the medium.

L26 ANSWER 14 OF 71 MEDLINE on STN

ACCESSION NUMBER: 90172183 MEDLINE

DOCUMENT NUMBER: PubMed ID: 2625666

TITLE: Sequence-directed DNA bending upstream of the **streptokinase** promoter.

AUTHOR: Muller J; Malke H

CORPORATE SOURCE: Akademie der Wissenschaften der DDR.

SOURCE: Journal of basic microbiology, (1989) 29 (9) 611-6.

Journal code: 8503885. ISSN: 0233-111X.

PUB. COUNTRY: GERMANY, EAST: German Democratic Republic

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199004

ENTRY DATE: Entered STN: 19900601

Last Updated on STN: 19900601

Entered Medline: 19900409

AB A 450-base pair (bp) **Hin**FI restriction fragment from the chromosome of *Streptococcus equisimilis* H46A contains the early coding region of the **streptokinase** gene (**sk**c), the **sk**c promoter, and a stretch of DNA 5' to the--35 region of the **sk**c promoter. Two-dimensional polyacrylamide (PA) gel electrophoresis at two different temperatures showed that this fragment migrates anomalously slowly on PA gels, suggesting the existence of a bent DNA conformation. Inspection of the nucleotide sequence confirmed this suggestion by revealing numerous oligomeric dA.dT tracts, some of which are in phase with the helix screw. Computer analysis of the sequence predicted the existence of two bending loci, one of which is located upstream of the **sk**c promoter. In addition to showing DNA bending, the 450-bp **Hin**FI fragment contains multiple 13-bp sequences homologous to the *Escherichia coli* integration host factor DNA-binding consensus sequence. Insertion of IS1 into a site immediately upstream of the--35 region decreased the **expression** level of **sk**c in *E. coli*, suggesting that DNA conformation upstream of the promoter has a role in **sk**c **expression**.

L26 ANSWER 15 OF 71 MEDLINE on STN

ACCESSION NUMBER: 88302119 MEDLINE

DOCUMENT NUMBER: PubMed ID: 3043172

TITLE: Tripartite **streptokinase** gene fusion vectors for gram-positive and gram-negative procaryotes.

AUTHOR: Klessen C; Schmidt K H; Ferretti J J; Malke H

CORPORATE SOURCE: Academy of Sciences of the GDR, Central Institute of Microbiology and Experimental Therapy, Jena.

SOURCE: Molecular & general genetics : MGG, (1988 May) 212 (2)

295-300.

Journal code: 0125036. ISSN: 0026-8925.

PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198809

ENTRY DATE: Entered STN: 19900308

Last Updated on STN: 19900308

Entered Medline: 19880921

AB A specific 1,596 bp HincII fragment ('*skc*') from the chromosome of *Streptococcus equisimilis* contains an active streptokinase (SK) gene (*skc*) lacking, in addition to the expression signals, codons 1 through 39 of wild-type *skc* but retaining the remainder of the *skc* coding sequence together with the transcription terminator. Using this fragment as an indicator gene, we constructed two types of vectors which in appropriate hosts resulted in the synthesis of SK fusion proteins after insertional activation of '*skc*'. The first type are open reading frame (ORF) vectors in which '*skc*' was inserted into pUC18 out of frame with respect to lacZ', thus conferring an SK-negative phenotype. Any DNA fragments representing ORFs inserted between the lacZ' expression signals and '*skc*' such that the *skc* reading frame was restored resulted in the production of tripartite proteins which exhibited SK activity. The second type of vector, which functioned in both gram-positive and gram-negative bacteria, used the streptococcal *speA* expression and secretion signals in front of the ORF to activate '*skc*' insertionally. Using a large fragment from the chymosin gene as the target sequence, the usefulness of these vectors for studying foreign gene expression in streptococci as well as *Escherichia coli* was demonstrated.

L26 ANSWER 16 OF 71 MEDLINE on STN

ACCESSION NUMBER: 84221999 MEDLINE

DOCUMENT NUMBER: PubMed ID: 6374659

TITLE: Streptokinase: cloning, expression, and excretion by *Escherichia coli*.

AUTHOR: Malke H; Ferretti J J

CONTRACT NUMBER: AI 9304 (NIAID)

SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1984 Jun) 81 (11) 3557-61.  
Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198407

ENTRY DATE: Entered STN: 19900320

Last Updated on STN: 19970203

Entered Medline: 19840724

AB Genomic DNA from *Streptococcus equisimilis* strain H46A was cloned in *Escherichia coli* by using the bacteriophage lambda replacement vector L47 and an in vitro packaging system. A casein/plasminogen overlay technique was used to screen the phage bank for recombinants carrying the streptokinase gene (*skc*). The gene was present with a frequency of 1 in 836 recombinants, and 10 independent clones containing *skc* were isolated and physically characterized. One recombinant clone was used to subclone *skc* in *E. coli* plasmid vectors. Plasmid pMF2 [10.4 kilobases (kb)] consisting of pACYC184 with a 6.4-kb H46A DNA fragment in the EcoRI site and pMF5 (6.9 kb) carrying a 2.5-kb fragment in the Pst I site of pBR322 were among the recombinant plasmids determining

streptokinase production in three different E. coli host strains. Expression of skc was independent of its orientation in either vector, indicating that its own promoter was present and functional in E. coli. However, expression in pBR322 was more efficient in one orientation than in the other, suggesting that one or both of the bla gene promoters contributed to skc expression. Several lines of evidence, including proof obtained by the immunodiffusion technique, established the identity of E. coli streptokinase. Testing cell-free culture supernatant fluids, osmotic shock fluids, and sonicates of osmotically shocked cells for streptokinase activity revealed the substance to be present in all three principal locations, indicating that E. coli cells were capable of releasing substantial amounts of streptokinase into the culture medium.

L26 ANSWER 17 OF 71 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2005441717 EMBASE  
 TITLE: Heberkinasa: Recombinant streptokinase [9].  
 AUTHOR: Hernandez L.; Martinez Y.; Quintana M.; Besada V.; Martinez E.  
 CORPORATE SOURCE: L. Hernandez, Production Division, Centro de Ingenieria Genetica Y Biotecnologia, Ave 31 e/ 158 y 190, Cubanacan, Playa, Habana 0600, Cuba. luciano.hernandez@cigb.edu.cu  
 SOURCE: European Heart Journal, (2005) Vol. 26, No. 16, pp. 1691.  
 Refs: 4  
 ISSN: 0195-668X CODEN: EHJODF  
 COUNTRY: United Kingdom  
 DOCUMENT TYPE: Journal; Letter  
 FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery  
 030 Pharmacology  
 037 Drug Literature Index  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 20051020  
 Last Updated on STN: 20051020

L26 ANSWER 18 OF 71 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2002110457 EMBASE  
 TITLE: Can imperfections help to improve bioreactor performance?.  
 AUTHOR: Patnaik P.R.  
 CORPORATE SOURCE: P.R. Patnaik, Institute of Microbial Technology, Sector 39-A, Chandigarh-160 036, India. pratap@imtech.res.in  
 SOURCE: Trends in Biotechnology, (1 Apr 2002) Vol. 20, No. 4, pp. 135-137.  
 Refs: 22  
 ISSN: 0167-7799 CODEN: TRBIDM  
 PUBLISHER IDENT.: S 0167-7799(01)01922-9  
 COUNTRY: United Kingdom  
 DOCUMENT TYPE: Journal; General Review  
 FILE SEGMENT: 004 Microbiology  
 027 Biophysics, Bioengineering and Medical Instrumentation  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English  
 ENTRY DATE: Entered STN: 20020404  
 Last Updated on STN: 20020404

AB Pilot-scale and larger bioreactors differ from small laboratory-scale reactors in terms of a greater occurrence of noise and incomplete mixing of the broth. Conventional control tries to induce good mixing and to filter out the noise as completely as possible. As such an 'ideal' operation is difficult to achieve, recent work has tried to exploit the non-ideal features to improve the performance. Using artificial neural

networks, the degree of mixing, the extent of filtering of noise and the distribution of plasmid copy number (in a recombinant fermentation) can be controlled effectively on-line. This strategy generates better productivities than well-mixed noise-free operations, which suggests that deviations from ideal behaviour should be gainfully harnessed and not suppressed.

L26 ANSWER 19 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:96119 BIOSIS  
DOCUMENT NUMBER: PREV200000096119  
TITLE: Two streptokinase genes are expressed with different solubility in *Escherichia coli* W3110.  
AUTHOR(S): Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis  
CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba  
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.  
CODEN: BILED3. ISSN: 0141-5492.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 15 Mar 2000  
Last Updated on STN: 3 Jan 2002

AB The streptokinase (SK) gene from *S. equisimilis* H46A (ATCC 12449) was cloned in *E. coli* W3110 under the control of the tryptophan promoter. The recombinant SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from *S. equisimilis* (ATCC 9542) which had been cloned in *E. coli* W3110 by using similar expression vector and cell growth conditions, and occurred in the form of inclusion bodies.

L26 ANSWER 20 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:88244 BIOSIS  
DOCUMENT NUMBER: PREV200000088244  
TITLE: Hydrodynamic radii of native and denatured proteins measured by pulse field gradient NMR techniques.  
AUTHOR(S): Wilkins, Deborah K.; Grimshaw, Shaun B.; Receveur, Veronique; Dobson, Christopher M.; Jones, Jonathan A.; Smith, Lorna J. [Reprint author]  
CORPORATE SOURCE: Oxford Centre for Molecular Sciences, New Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QT, UK  
SOURCE: Biochemistry, (Dec. 14, 1999) Vol. 38, No. 50, pp. 16424-16431. print.  
CODEN: BICHAW. ISSN: 0006-2960.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 10 Mar 2000  
Last Updated on STN: 3 Jan 2002

AB Pulse field gradient NMR methods have been used to determine the effective hydrodynamic radii of a range of native and nonnative protein conformations. From these experimental data, empirical relationships between the measured hydrodynamic radius ( $R_h$ ) and the number of residues in the polypeptide chain ( $N$ ) have been established; for native folded proteins  $R_h = 4.75N^{0.29}$ ANG and for highly denatured states  $R_h = 2.21N^{0.57}$ ANG. Predictions from these equations agree well with experimental data from dynamic light scattering and small-angle X-ray or neutron scattering studies reported in the literature for proteins ranging in size

from 58 to 760 amino acid residues. The predicted values of the hydrodynamic radii provide a framework that can be used to analyze the conformational properties of a range of nonnative states of proteins. Several examples are given here to illustrate this approach including data for partially structured molten globule states and for proteins that are unfolded but biologically active under physiological conditions. These reveal evidence for significant coupling between local and global features of the conformational ensembles adopted in such states. In particular, the effective dimensions of the polypeptide chain are found to depend significantly on the level of persistence of regions of secondary structure or features such as hydrophobic clusters within a conformational ensemble.

L26 ANSWER 21 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:317596 BIOSIS

DOCUMENT NUMBER: PREV199800317596

TITLE: Effect of signal peptide changes on the extracellular processing of **streptokinase** from *Escherichia coli*: Requirement for secondary structure at the cleavage junction.

AUTHOR(S): Pratap, J.; Dikshit, K. L. [Reprint author]

CORPORATE SOURCE: Inst. Microbial Technology, Sector 39-A, Chandigarh 160 036, India

SOURCE: Molecular and General Genetics, (May, 1998) Vol. 258, No. 4, pp. 326-333. print.

CODEN: MGGEAE. ISSN: 0026-8925.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 22 Jul 1998

Last Updated on STN: 10 Sep 1998

AB **Streptokinase** (SK), an extracellular protein from *Streptococcus equisimilis*, is secreted post-translationally by *Escherichia coli* using both its native and *E. coli*-derived transport signals. In this communication we report that cleavage specificity of signal peptidase I, and thus efficiency of secretion, varies in *E. coli* when SK export is directed by different transport signals. The native (+ 1) N-terminus of mature SK was retained when it was transported under the control of its own, PelB or LamB signal peptide. However, when translocation of SK was controlled by the OmpA or Male signal peptide, Ala2 of mature SK was preferred as a cleavage site for the pre-SK processing. Our results indicate that compatibility of the leader peptide with the mature sequences of SK, which fulfils the requirement for a given secondary structure within the cleavage region, is essential for maintaining the correct processing of pre-SK. An OmpA-SK fusion, which results in the deletion of two N-terminal amino acid residues of mature SK, was further studied with respect to the recognition of alternative cleavage site in *E. coli*. The alanine at +2 in mature SK was changed to glycine or its relative position was changed to +3 by introducing a methionine residue at the +1 position. Both alterations resulted in the correct cleavage of pre-SK at the original OmpA fusion site. In contrast, introduction of an additional alanine at +4, creating three probable cleavage sites (Ala-x-Ala-x-Ala-x-Ala), resulted in the recognition of all three target sites for cleavage, with varying efficiency. The results indicate that the nature of the secondary structure generated at the cleavage junction of pre-SK, resulting from the fusion of different signal peptides, modulates the cleavage specificity of signal peptidase I during extracellular processing of SK. Based on these findings it is proposed that flexibility in the interaction of the active site of signal peptidase I with the cleavage sites of signal peptides may occur when it encounters two or more juxtaposed cleavage sites. Preference for one cleavage site over another, then, may depend on fulfillment of secondary structure requirements in the vicinity of the pre-protein cleavage junction.

L26 ANSWER 22 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
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ACCESSION NUMBER: 1995:547506 BIOSIS  
DOCUMENT NUMBER: PREV199698561806  
TITLE: High-level **expression** and secretion of  
**streptokinase** in *Escherichia coli*.  
AUTHOR(S): Ko, Jae Hyeong; Park, Do Deun; Kim, Il Chul; Lee, Si  
Hyoung; Byun, Si Myung [Reprint author]  
CORPORATE SOURCE: Dep. BioSci., Korea Advanced Inst. Sci. Technol., 373-1,  
Kusung-dong, Yusung-ku, Taejeon 305-701, South Korea  
SOURCE: Biotechnology Letters, (1995) Vol. 17, No. 10, pp.  
1019-1024.  
CODEN: BILED3. ISSN: 0141-5492.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 31 Dec 1995  
Last Updated on STN: 31 Dec 1995

AB The high-level **expression** plasmid for **streptokinase**,  
pSK100, has been constructed. It contains a tac promoter, an ompA signal  
sequence, a **streptokinase** structural gene(**skc**) and a  
rrnBT1T2 transcription terminator. *E. coli* JM 109 carrying  
pSK100 produced about 5,000IU of **streptokinase** per 1 ml of  
LB-ampicillin media. About 95% of the **expressed**  
**streptokinase** was secreted into the periplasmic and extracellular  
fractions. The **recombinant streptokinase** in high  
yield and purity may be a potential alternative source for the therapeutic  
agent.

L26 ANSWER 23 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1992:340953 BIOSIS  
DOCUMENT NUMBER: PREV199243030503; BR43:30503  
TITLE: **STREPTOKINASE** MUTATIONS AFFECTING **SKC**  
**EXPRESSION** IN HOMOLOGOUS AND HETEROLOGOUS HOSTS.  
AUTHOR(S): MECHOLD U [Reprint author]; MULLER J; MALKE H  
CORPORATE SOURCE: CENTRAL INST MICROBIOL EXP THERAPY, JENA D-6900, GER  
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1992) pp.  
336-338. OREFICI, G. (ED.). ZENTRALBLATT FUER BAKTERIOLOGIE  
SUPPLEMENT, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND  
STREPTOCOCCAL INFECTIONS; (INTERNATIONAL JOURNAL OF MEDICAL  
MICROBIOLOGY, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND  
STREPTOCOCCAL INFECTIONS); XI LANCEFIELD INTERNATIONAL  
SYMPOSIUM ON STREPTOCOCCI AND STREPTOCOCCAL DISEASES,  
SIENA, ITALY, SEPTEMBER 10-14, 1990. XIX+569P. GUSTAV  
FISCHER VERLAG: STUTTGART, GERMANY; NEW YORK, NEW YORK,  
USA. ILLUS.  
Publisher: Series: Zentralblatt fuer Bakteriologie  
Supplement.  
ISSN: 0941-018X. ISBN: 3-437-11362-3, 1-56081-333-4.  
DOCUMENT TYPE: Book  
Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 16 Jul 1992  
Last Updated on STN: 16 Jul 1992

L26 ANSWER 24 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1991:513401 BIOSIS  
DOCUMENT NUMBER: PREV199141114116; BR41:114116  
TITLE: **EXPRESSION** AND PROPERTIES OF HYBRID  
**STREPTOKINASES** EXTENDED BY AMINO-TERMINAL  
PLASMINOGEN KRINGLE DOMAINS.

AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J  
 CORPORATE SOURCE: DEP MICROBIOL IMMUNOL, UNIV OKLA HEALTH SCI CENTER,  
 OKLAHOMA CITY, OKLA 73190, USA  
 SOURCE: (1991) pp. 184-189. DUNNY, G. M., P. P. CLEARY AND L. L.  
 MCKAY (ED.). GENETICS AND MOLECULAR BIOLOGY OF  
 STREPTOCOCCI, LACTOCOCCI, AND ENTEROCOCCI; THIRD  
 INTERNATIONAL ASM (AMERICAN SOCIETY FOR MICROBIOLOGY)  
 CONFERENCE, MINNEAPOLIS, MINNESOTA, USA, JUNE 6-9, 1990.  
 VIII+310P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON,  
 D.C., USA. ILLUS.  
 ISBN: 1-55581-034-9.  
 DOCUMENT TYPE: Book  
 Conference; (Meeting)  
 FILE SEGMENT: BR  
 LANGUAGE: ENGLISH  
 ENTRY DATE: Entered STN: 14 Nov 1991  
 Last Updated on STN: 14 Nov 1991

L26 ANSWER 25 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
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ACCESSION NUMBER: 1987:499919 BIOSIS  
 DOCUMENT NUMBER: PREV198733127633; BR33:127633  
 TITLE: **STREPTOKINASE EXPRESSION OF ALTERED  
 FORMS.**  
 AUTHOR(S): MALKE H [Reprint author]; LORENZ D; FERRETTI J J  
 CORPORATE SOURCE: ACAD SCI GER DEMOCRATIC REPUBLIC, CENT INST MICROBIOL AND  
 EXP THERAPY, DDR-69 JENA, GDR  
 SOURCE: (1987) pp. 143-149. FERRETTI, J. J. AND R. CURTISS, III  
 (ED.). STREPTOCOCCAL GENETICS; SECOND ASM (AMERICAN SOCIETY  
 FOR MICROBIOLOGY) CONFERENCE, MIAMI, FLORIDA, USA, MAY  
 21-24, 1986. VIII+300P. AMERICAN SOCIETY FOR MICROBIOLOGY:  
 WASHINGTON, D.C., USA. ILLUS.  
 ISBN: 0-914826-93-X.  
 DOCUMENT TYPE: Book  
 Conference; (Meeting)  
 FILE SEGMENT: BR  
 LANGUAGE: ENGLISH  
 ENTRY DATE: Entered STN: 27 Nov 1987  
 Last Updated on STN: 27 Nov 1987

L26 ANSWER 26 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
 STN

ACCESSION NUMBER: 1987:277792 BIOSIS  
 DOCUMENT NUMBER: PREV198784018831; BA84:18831  
 TITLE: **MOLECULAR CLONING OF STREPTOKINASE GENE  
 FROM STREPTOCOCCUS-EQUISIMILIS AND ITS  
 EXPRESSION IN ESCHERICHIA-COLI.**  
 AUTHOR(S): ROH D C [Reprint author]; KIM J H; PARK S K; LEE J W; BYRUN  
 S M  
 CORPORATE SOURCE: DEP BIOLOGICAL SCIENCE AND ENGINEERING, KOREA ADVANCED INST  
 SCIENCE AND TECHNOLOGY KAIST , PO BOX 150 CHONGRYANG, SEOUL  
 131, KOREA  
 SOURCE: Korean Biochemical Journal, (1986) Vol. 19, No. 4, pp.  
 391-398.  
 CODEN: KBCJAK. ISSN: 0368-4881.  
 DOCUMENT TYPE: Article  
 FILE SEGMENT: BA  
 LANGUAGE: ENGLISH  
 ENTRY DATE: Entered STN: 19 Jun 1987  
 Last Updated on STN: 19 Jun 1987

AB The streptococcal genomic DNA digested with Pst I was cloned in  
 E. coli HB101. The overlay technique of casein/plasminogen was  
 used to screen the clones for recombinants carrying  
 the streptokinase gene. The insert size of the plasmid carrying

the **streptokinase** gene was a 2.5, 4.3, and 5.8 Kb, respectively. The restriction maps of all three hybrid plasmids were constructed by digestion with Pst I, Pvu II, Sal I, Hind III, Ava I, BamH I, and Cla I. For the identification of **cloned gene, streptokinase** was highly purified from *S. equisimilis* by the methods of gel chromatography and isoelectric focusing and rabbits were immunized with this purified **streptokinase**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established that the enzyme from *E. coli* was identical to that from *S. equisimilis*. In the *E. coli* cell culture, we found the activity of **streptokinase** in all three principal locations of the cell. More than 50% were existed in the intracellular space.

L26 ANSWER 27 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1984:100214 BIOSIS  
DOCUMENT NUMBER: PREV198427016706; BR27:16706  
TITLE: HYBRIDIZATION OF A CLONED GROUP C STREPTOCOCCAL STREPTO KINASE GENE WITH DNA FROM OTHER STREPTOCOCCAL SPECIES.  
AUTHOR(S): HUANG T-T [Reprint author]; WEEKS C R; MALKE H; FERRETTI J J  
CORPORATE SOURCE: UNIV OKLA HEALTH SCI CENT, OKLAHOMA CITY, OKLA, USA  
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D75.  
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.  
ABSTR ANNU MEET AM SOC MICROBIOL.  
CODEN: ASMACK. ISSN: 0094-8519.  
DOCUMENT TYPE: Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH

L26 ANSWER 28 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1984:92832 BIOSIS  
DOCUMENT NUMBER: PREV198427009324; BR27:9324  
TITLE: STREPTO KINASE CLONING EXPRESSION AND EXCRETION BY ESCHERICHIA-COLI.  
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J  
CORPORATE SOURCE: ACAD SCI GDR, JENA, GDR  
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D97.  
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.  
ABSTR ANNU MEET AM SOC MICROBIOL.  
CODEN: ASMACK. ISSN: 0094-8519.  
DOCUMENT TYPE: Conference; (Meeting)  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH

L26 ANSWER 29 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1994-07329 BIOTECHDS  
TITLE: DNA encoding a plasminogen binding protein;  
**recombinant streptokinase** fragment  
production using new vector plasmid pMAL and a monoclonal antibody for use in myocardial infarction therapy  
PATENT ASSIGNEE: Gen.Hosp.Boston; Univ.Harvard  
PATENT INFO: WO 9407992 14 Apr 1994  
APPLICATION INFO: WO 1993-US9502 5 Oct 1993  
PRIORITY INFO: US 1993-128299 29 Sep 1993; US 1992-956692 5 Oct 1992  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 1994-135561 [16]



AB DNA (I) encoding a **streptokinase** fragment (II) of residues 14-414 of a disclosed protein sequence is claimed. (II) does not contain residues 244-352, but may contain residues 1-352, 120-352, 244-414 or 244-352 of the protein sequence. Also claimed are: (1) an **expression** vector containing (I); (2) a host cell transformed with the vector of (1); (3) (II) encoded by (I); (4) detecting plasminogen in a biological sample by contacting the sample with (II) and detecting any (II)-plasminogen complex formed; (5) a method for assaying (II) for antigenicity involving contacting (II) with a monoclonal antibody specific for a distinct epitope of **streptokinase** and determining whether the fragments bind to the MAb and, optionally, also whether the fragments can activate plasminogen in the presence of the MAb; (6) a method for myocardial infarction therapy involving administering (II) to a patient; and (7) a monoclonal antibody specific for a distinct epitope of **streptokinase**. In an example, (II) genes from *Streptococcus equisimilis* were fused with maltose binding protein genes and **expressed** in *Escherichia coli* using plasmid pMAL. (62pp)

L26 ANSWER 30 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1992-13545 BIOTECHDS

TITLE: High level **expression** of **streptokinase** in  
*Escherichia coli*;

gene cloning, **expression** and  
purification of thrombolytic protein

AUTHOR: Estrada M P; Hernandez L; Perez A; Rodriguez P; \*de la Fuente  
J; Herrera L

LOCATION: Mammalian Cell Genetics Division, Centro de Ingenieria  
Genetica y Biotecnologia, P.O. Box 6162, Havana 6, Cuba.

SOURCE: Bio/Technology; (1992) 10, 10, 1138-42  
CODEN: BTCHDA

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **streptokinase** (SK) gene was isolated by the polymerase chain reaction from *Streptomyces equisimilis* ATCC 9542. The 5-amplification primer introduced an ATG codon for translation initiation in *Escherichia coli*. The amplified fragment, which lacked the signal peptide sequence, was digested with BamHI, inserted into vector plasmid pTrp (to obtain plasmid pEKG-3 containing the SK gene under the control of a trp promoter), and used to transform *E. coli* HB101 cells. The DNA sequence of the SK gene region contained 5 differences at the amino acid level with respect to the reported SK protein. Plasmid pEKG-3 was introduced into *E. coli* K-12 strain W3110 for **expression**. The trp promoter was induced, and maximal SK **expression** was obtained after 14 hr, at which time the plasmid copy number reached 420 copies/cell. The **recombinant** SK was found in the cell cytosol and constituted 25% of total cell protein. It was purified by affinity chromatography using acylated human plasminogen coupled to Sepharose-4B, and ionexchange chromatography on DEAE-Sephacel. The **recombinant** product and natural SK had equivalent biological activities. (38 ref)

L26 ANSWER 31 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1992-03808 BIOTECHDS

TITLE: Method for the isolation and **expression** of a gene  
encoding **streptokinase**;  
*Streptococcus equisimilis* gene cloning  
and vector plasmid pEKG3, plasmid pPESKC-4 and plasmid  
pPISKC-6 **expression** in *Escherichia coli*  
or *Pichia pastoris*

PATENT ASSIGNEE: Cent.Ing.Genet.Biotecnol.

PATENT INFO: AU 9178101 28 Nov 1991

APPLICATION INFO: AU 1991-78101 31 May 1991

PRIORITY INFO: CU 1990-90 23 May 1990

DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 1992-024716 [04]

AB A new method for the isolation and **expression** of a gene, **SKC-2**, encoding *Streptococcus equisimilis* C (ATCC 9542) **streptokinase** comprises (i) gene amplification from synthetic oligonucleotides SK1, SK2 and SK3 (specified DNA sequence), (ii) **cloning** **SKC-2** in bacteria (preferably *Escherichia coli*) with or without a signal peptide; and (iii) intra- or extracellular **expression** in yeast (preferably *Pichia pastoris*), with the transformed microorganism displaying a high stability and level of **expression**. The following are also claimed: (1) plasmid pEKG3 containing **SKC-2** inserted between the trp promoter and the phage T4 terminator for **expression** in bacteria; (2) plasmid pPESKC-4 and plasmid pPISKC-6, obtained by insertion of **SKC-2** in the yeast **expression** vectors plasmid pPS-7 and plasmid pNAO, respectively, for extra- or intracellular **expression**; (3) transformed microorganisms displaying high levels of **SKC-2** gene **expression**, good viability and cellular stability; (4) the product resulting from **expression** of the **SKC-2** gene in bacteria and yeast; (5) **recombinant** DNA comprising the **SKC-2** DNA sequence; and (6) the **expression** product of the **recombinant** DNA. (28pp)

L26 ANSWER 32 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1991-01878 BIOTECHDS

TITLE: Constructing vector for detecting **expression** of foreign genes;  
by inserting element containing **expression** unit, **streptokinase** gene and restriction sites, allowing in frame gene insertion; pro-chymosin, beta-galactosidase production

PATENT ASSIGNEE: Akad.Wiss.DDR  
PATENT INFO: DD 279900 20 Jun 1990  
APPLICATION INFO: DD 1987-306609 3 Sep 1987  
PRIORITY INFO: DD 1987-306609 3 Sep 1987  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
OTHER SOURCE: WPI: 1990-342373 [46]

AB Construction of vectors for detecting heterologous gene **expression** comprises: i. incorporating (in order from N terminus) into a **cloning** vector, a polylinker or restriction site (RS1); **expression** or **expression** secretion unit (*Escherichia coli* lac operon or exotoxin A gene of phage T12 from *Streptococcus pyogenes*; polylinker or restriction site (RS2); **streptokinase** (SK) structural gene (from *Streptococcus equisimilis* H46A, particularly a 1596 bp HindIII fragment from plasmid pMF5), a polylinker or restriction site (RS3); ii. inserting a foreign gene, X, without a promoter into polylinker or RS2; iii. the resulting detection vector, encoding for an X-SK fusion product, is used to transform microbial receptor cells; and iv. subjecting **recombinant** clones to a plasminogen-milk agar (PMA) overlaying test. Preferably, the vector is a bacterial plasmid or an M13 *E. coli* phage vector. X is a pro-chymosin gene (plasmid pHRW400 or plasmid pHRW500), human interferon-alpha-1, or beta-galactosidase (EC-3.2.1.23, from a pUC plasmid). Recipients are *E. coli* JM101, *Streptococcus sanguis* Challis 6 or *Streptococcus lactis* MG1363. (14pp)

L26 ANSWER 33 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1990-02600 BIOTECHDS

TITLE: Site-specific alteration of Gly-24 in **streptokinase**  
: its effect on plasminogen activation;

site-directed mutagenesis effect on plasminogen-activator activity; gene cloning and expression in *Escherichia coli*

AUTHOR: Lee B R; Park S K; Kim J H; \*Byun S M  
LOCATION: Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), P.O. Box 150, Cheongryang, Seoul, Korea.  
SOURCE: Biochem.Biophys.Res.Communic.; (1989) 165, 3, 1085-90  
CODEN: BBRC A9  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Oligonucleotide site-directed mutagenesis was performed to replace Gly-24 of *Streptococcus equisimilis* (ATCC 9542) streptokinase with His, Glu, or Ala. The streptokinase gene was cloned, subjected to mutagenesis for removal of the *RsaI* site, cloned into vector plasmid pKS601 under the control of the *trp* promoter and used to transform *Escherichia coli* C600. The recombinant proteins were purified by DEAE-cellulose and Sephadex-G150 chromatography. Substitutions with either His or Glu gave almost complete loss of streptokinase activity but streptokinase replaced with Ala retained its activity. Although streptokinases with His-24 or Glu-24 bound normally to human plasminogen, they did not generate active plasmin, whereas those with Ala-24 or Gly-24 generated active plasmin. The results indicate that the small, uncharged alkyl group side chain on the 24th amino acid residue of streptokinase is indispensable for the activity of the human plasminogen-streptokinase complex. A charged amino acid in position 24 disrupts beta-sheet formation and prevents streptokinase from adopting the orientation required for plasminogen activation. (26 ref)

L26 ANSWER 34 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1985-08513 BIOTECHDS  
TITLE: Production of streptokinase;

by cultivation of *Escherichia coli* ATCC 39613 containing recombinant plasmid PMF1

PATENT ASSIGNEE: Phillips-Petrol.  
PATENT INFO: AU 8433859 18 Apr 1985  
APPLICATION INFO: AU 1984-33859 5 Oct 1984  
PRIORITY INFO: US 1984-585417 2 Mar 1984; DD 1983-255523 10 Oct 1983  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 1985-135032 [23]

AB A recombinant vector plasmid PMF1 for the transformation of a host to produce streptokinase is new. The vector contains a polydeoxyribonucleotide fragment insert which codes for the synthesis and secretion of streptokinase. The transformant microorganism is preferably *Escherichia coli* HB101 and the vector, plasmid pBR322. The fragment coding for streptokinase synthesis and secretion is derived from a microorganism of the genus *Streptococcus*, especially *Streptococcus equisimilis* strain H46A and may have restriction endonuclease cleavage sites at the termini e.g. it has 7400 bp and the cleavage sites are for *HindIII*. The recombinant vector is obtained by digestion of a vector with a restriction endonuclease to give linear DNA. This DNA is ligated to the streptokinase fragment to give the recombinant. This fragment is obtained by digestion of *Streptococcus equisimilis* with the same restriction endonuclease, especially *Pst I*, as is used to digest the initial vector. Streptokinase can be produced and isolated for use as a thrombolytic agent to facilitate the in vivo lysis or dissolution of blood clots. (28pp)

L26 ANSWER 35 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1984-03868 BIOTECHDS

TITLE: Streptokinase: cloning expression  
and excretion by E.coli;  
using Streptococcus equisimilis genomic DNA  
(conference abstract)

AUTHOR: Malke H; Ferretti J J

LOCATION: Acad. Sci. GDR, Jena, DDR.

SOURCE: Abstr.Annu.Meet.Am.Soc.Microbiol; (1984) 84 Meet., 67

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Genomic DNA from Streptococcus equisimilis H46A (group C) was cloned into Escherichia coli using the lambda replacement vector L47 and an in vitro packaging system. The phage bank was screened for recombinants containing the streptokinase (skc) gene by the casein-plasminogen overlay technique. 10 Independent clones containing the skc gene were isolated and one was used to subclone the skc gene into E.coli plasmid vectors pBR322 and pACYC184. Plasmid pMF2 and pMF5 were among the recombinant plasmids determining streptokinase production in 3 different E. coli host strains. Expression of skc was independent of its orientation in either vector, indicating that its own promoter was present and functional in E.coli. Analysis of cell free culture supernatant fluids, osmotic shockates, and sonicates of osmotically shocked cells for streptokinase activity revealed the substance to be present in all 3 locations, indicating that E. coli cells were capable of releasing substantial amounts of streptokinase into the culture medium. (0 ref)

L26 ANSWER 36 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:656911 SCISEARCH

THE GENUINE ARTICLE: 837ZE

TITLE: Control of streptokinase gene expression  
in group A & C streptococci by two-component regulators

AUTHOR: Malke H (Reprint); Steiner K

CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany  
hmalke@imb-jena.de

COUNTRY OF AUTHOR: Germany

SOURCE: INDIAN JOURNAL OF MEDICAL RESEARCH, (MAY 2004) Vol. 119, Supp. [S], pp. 48-56.  
ISSN: 0971-5916.

PUBLISHER: INDIAN COUNCIL MEDICAL RES, PO BOX 4911 ANSARI NAGAR, NEW DELHI 110029, INDIA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 23

ENTRY DATE: Entered STN: 13 Aug 2004  
Last Updated on STN: 13 Aug 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Background & objectives: Group A streptococci (GAS) and human isolates of group C streptococci (GCS) have the stable capacity to produce the plasminogen activator streptokinase, albeit with varying efficiency. This property is subject to control by two two-component regulatory systems, FasCAX and CovRS, which act as activator and repressor, respectively. The present work aims at balancing these opposing activities in GAS and GCS, and at clarifying the phylogenetic position of the FasA response regulator, the less understood regulator of the two systems.

Methods: The GCS strain H46A and GAS strain NZ131 were used. Escherichia coli JM 109 was used as host for plasmid construction. Streptokinase activity of various wild type and mutant strains was measured. Phylogenetic trees of streptococcal FasA

homologues were established.

Results: The **streptokinase** activities of the GAS strain NZ131 and the GCS strain H46A were attributable to more efficient CovR repressor action in NZ131 than in H46A. The FasA activator, on the other hand, functioned about equally efficient in the two strains. Phylogenetically, FasA homologues clustered distinctly in the proposed FasA-BlpR-Come family of streptococcal response regulators and used the LytTR domain for DNA binding.

Interpretation & conclusion: Assessing the apparent **streptokinase** activity of streptococcal strains require the dissection of the activities of the cov and fas systems. Although experimental evidence is still missing, FasA is closely related to a widely distributed family of streptococcal response regulators that is involved in behavioral processes, such as quorum sensing.

L26 ANSWER 37 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:69727 SCISEARCH

THE GENUINE ARTICLE: 763KK

TITLE: **Streptokinase** - a clinically useful thrombolytic agent

AUTHOR: Banerjee A; Chisti Y; Banerjee U C (Reprint)

CORPORATE SOURCE: Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Sector 67, Mohali 160062, Punjab, India (Reprint); Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Mohali 160062, Punjab, India; Massey Univ, Inst Technol & Engrn, Palmerston North, New Zealand

COUNTRY OF AUTHOR: India; New Zealand

SOURCE: BIOTECHNOLOGY ADVANCES, (FEB 2004) Vol. 22, No. 4, pp. 287-307.

ISSN: 0734-9750.

PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.

DOCUMENT TYPE: General Review; Journal

LANGUAGE: English

REFERENCE COUNT: 171

ENTRY DATE: Entered STN: 30 Jan 2004

Last Updated on STN: 30 Jan 2004

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A failure of hemostasis and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infraction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and **streptokinase**. This review deals with **streptokinase** as a clinically important and cost-effective plasminogen activator. The aspects discussed include: the mode of action; the structure and structure-function relationships; the structural modifications for improving functionality; **recombinant streptokinase**; microbial production; and recovery of this protein from crude broths. (C) 2003 Published by Elsevier Inc.

L26 ANSWER 38 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:148580 SCISEARCH

THE GENUINE ARTICLE: 285UW

TITLE: Allele substitution of the **streptokinase** gene reduces the nephritogenic capacity of group A streptococcal strain NZ131

AUTHOR: Nordstrand A (Reprint); McShan W M; Ferretti J J; Holm S E; Norgren M

CORPORATE SOURCE: Umea Univ, Dept Clin Bacteriol, S-90185 Umea, Sweden (Reprint); Univ Oklahoma, Hlth Sci Ctr, Dept Microbiol & Immunol, Oklahoma City, OK 73190 USA

COUNTRY OF AUTHOR: Sweden; USA

SOURCE: INFECTION AND IMMUNITY, (MAR 2000) Vol. 68, No. 3, pp. 1019-1025.  
ISSN: 0019-9567.  
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 36  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB To investigate the role of allelic variants of **streptokinase** in the pathogenesis of acute poststreptococcal glomerulonephritis (APSGN), site-specific integration plasmids were constructed, which contained either the non-nephritis-associated **streptokinase** gene (skc5) from the group C streptococcal strain *Streptococcus equisimilis* H46A or the nephritis-associated **streptokinase** gene (ska1) from the group A streptococcal nephritogenic strain NZ131. The plasmids were introduced by electroporation and homologous recombination into the chromosome of an isogenic derivative of strain NZ131, in which the **streptokinase** gene had been deleted and which had thereby lost its nephritogenic capacity in a mouse model of APSGN. The introduction of a non-nephritis-associated allelic variant of **streptokinase** did not rescue the nephritogenic capacity of the strain. The mutant and the wild-type strains produced equivalent amounts of **streptokinase**. Complementation of the ska deletion derivative with the original ska allele reconstituted the nephritogenicity of wild-type NZ131. The findings support the hypothesis that the role of **streptokinase** in the pathogenesis of APSGN is related to the allelic variant of the protein.

L26 ANSWER 39 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:100732 SCISEARCH  
THE GENUINE ARTICLE: 277JH  
TITLE: Genetic organisation of the M protein region in human isolates of group C and G streptococci: two types of multigene regulator-like (mgrC) regions  
AUTHOR: Geyer A; Schmidt K H (Reprint)  
CORPORATE SOURCE: Univ Jena, Univ Hosp, Inst Med Microbiol, Semmelweisstr 4, D-07740 Jena, Germany (Reprint); Univ Jena, Univ Hosp, Inst Med Microbiol, D-07740 Jena, Germany  
COUNTRY OF AUTHOR: Germany  
SOURCE: MOLECULAR AND GENERAL GENETICS, (JAN 2000) Vol. 262, No. 6, pp. 965-976.  
ISSN: 0026-8925.  
PUBLISHER: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 47  
ENTRY DATE: Entered STN: 2000  
Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In addition to beta-haemolytic streptococci belonging to Lancefield group A (*Streptococcus pyogenes*, GAS), human isolates of group C (GCS) and group G (GGS) streptococci (*S. dysgalactiae* subsp. *equisimilis*;) have been implicated as causative agents in outbreaks of purulent pharyngitis, of wound infections and recently also of streptococcal toxic shock-like syndrome. Very little is known about the organisation of the genomic region in which the emm gene of GCS and GCS is located. We have investigated the genome sequences flanking the emm gene in GCS by sequencing neighbouring fragments obtained by inverse PCR. Our sequence data for GCS strains 25287 and H46A revealed two types of arrangement in the emm region, which differ significantly from the known types of mga

regulon in GAS. We named this segment of the genome mgrC (for multigene regulon-like segment in group C streptococci). In strains belonging to the first mgrC type (prototype strain 25287) the emm gene is flanked upstream by mgc, a gene that is 61% identical to the mga gene of GAS. A phylogenetic analysis of the deduced protein sequences showed that Mgc is related to Mga proteins of various types of GAS but forms a distinct cluster. Downstream of emm, the mgrC sequence region is bordered by rel. This gene encodes a protein that functions in the synthesis and degradation of guanosine 3',5' bipyrophosphate (ppGpp) during the stringent regulatory response to amino acid deprivation. In the second mgrC type (prototype strain H46A), the genes mgc and emm are arranged as in type 1. But an additional ORF (orf) is inserted in opposite orientation between emm and rel. This orf shows sequence homology to cpdB, which is present in various microorganisms and encodes 2',3' cyclo-nucleotide 2'-phosphodiesterase. PCR analysis showed that these two mgrC arrangements also exist in GGS. Our sequence and PCR data further showed that both types of mgrC region in GCS and GGS are linked via rel to the streptokinase region characterised recently in strain H46A. A gene encoding C5a peptidase, which is present at the 3' end of the mga regulon in GAS, was not found in the mgrC region identified in the GCS and GGS strains investigated here.

L26 ANSWER 40 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:666643 SCISEARCH  
 THE GENUINE ARTICLE: 231GM  
 TITLE: PauA: a novel plasminogen activator from Streptococcus uberis  
 AUTHOR: Rosey E L; Lincoln R A; Ward P N; Yancey R J; Leigh J A (Reprint)  
 CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint); Pfizer Inc, Div Cent Res, Anim Hlth Biol Discovery, Groton, CT 06340 USA  
 COUNTRY OF AUTHOR: England; USA  
 SOURCE: FEMS MICROBIOLOGY LETTERS, (1 SEP 1999) Vol. 178, No. 1, pp. 27-33.  
 ISSN: 0378-1097.  
 PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 20  
 ENTRY DATE: Entered STN: 1999  
 Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Chromosomal DNA from two geographically distinct isolates of Streptococcus uberis was used to clone the plasminogen activator in an active form in Escherichia coli. The cloned fragments from each strain contained four potential open reading frames (ORFs). That for the plasminogen activator encoded a protein of 286 amino acids (33.4 kDa) which is cleaved between residues 25 and 26 during secretion by S. uberis. The amino acid sequence of the mature protein showed only weak homology (23.5-28%) to streptokinase. The plasminogen activator gene, pauA, in S. uberis was located between two ORFs with high homology to the DNA mismatch repair genes, hexA and hexB, and not on a DNA fragment between the genes encoding an ATP binding cassette transporter protein (abc) and a protein involved in the formation and degradation of guanosine polyphosphates (rel) as is the case for streptokinase in other streptococci. (C) 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

L26 ANSWER 41 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:317401 SCISEARCH  
 THE GENUINE ARTICLE: 189BZ  
 TITLE: Hydrophobic interaction chromatography applied to purification of **recombinant streptokinase**  
 AUTHOR: Perez N (Reprint); Urrutia E; Camino J; Orta D R; Torres Y; Martinez Y; Cruz M; Alburquerque S; Gil M R; Hernandez L  
 CORPORATE SOURCE: Ctr Genet Engn & Biotechnol, Streptokinase Div, Havana, Cuba; Ctr Genet Engn & Biotechnol, Qual Control Div, Havana, Cuba  
 COUNTRY OF AUTHOR: Cuba  
 SOURCE: MINERVA BIOTECNOLOGICA, (DEC 1998) Vol. 10, No. 4, pp. 174-177.  
 ISSN: 1120-4826.  
 PUBLISHER: EDIZIONI MINERVA MEDICA, CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 22  
 ENTRY DATE: Entered STN: 1999  
 Last Updated on STN: 1999

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Background **Recombinant streptokinase (rSk)** is a streptococcal protein cloned in *E. coli*. (11) Several methods have been described for **streptokinase** purification: ion exchange chromatography, (12) affinity chromatography with canine plasmin (13) and chromatography on immobilized acylated human plasminogen. (14) Monoclonal antibodies anti-rSk immobilized to Sepharose (15) have been used too. Recently this protein was purified using HIC.  
 Methods. rSk (CIGB, Cuba) was produced by fermentation strain K12 of *E. coli*, (11) the protein was recovered after washed pellet, cellular disruption and solubilization. Several purification assays were done using TSK-Butyl (Tosohaas, Japan) as a support for hydrophobic interaction chromatography (HIC). The protein was loaded to the column with 1 M of ammonium sulfate before being washed using an elution gradient from 0.5 to 0 M of ammonium sulfate, in order to determine the elution point of the rSk.  
 Results. Wie could determine that this protein is partly hydrophobic, this determination was shown by analysis of its aminoacidic sequence. This protein has 415 aminoacids of which 36% are non polar. The absorption capacity for TSK Butyl 650 S varies from 15 to 20 mg/mL. The optimum elution point was obtained using 0.25 M of ammonium sulfate, the eluted material was obtained with a high level of purity (<1% of contaminants). The recovery of rSk was about 49% using the mean of five assays.  
 Conclusions. The experimental process evaluated could be efficiently inserted in a downstream process to obtain **recombinant streptokinase** highly purified as final preparation and good recovery.

L26 ANSWER 42 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:473042 SCISEARCH  
 THE GENUINE ARTICLE: ZV077  
 TITLE: The interaction of *Streptococcus dysgalactiae* with plasmin and plasminogen  
 AUTHOR: Leigh J A (Reprint); Hodgkinson S M; Lincoln R A  
 CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint)  
 COUNTRY OF AUTHOR: England  
 SOURCE: VETERINARY MICROBIOLOGY, (15 MAR 1998) Vol. 61, No. 1-2, pp. 121-135.



ISSN: 0378-1135.  
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
NETHERLANDS.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 29  
ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The activation of plasminogen and the binding of plasmin by bacteria may have many effects which promote infection. The occurrence of such activities in streptococci is well documented; however, these are yet to be demonstrated for *S. dysgalactiae*. Consequently, the ability of this bacterium to activate mammalian plasminogen and bind either plasmin or its zymogen was investigated. Activation of bovine plasminogen was dependant on both the strain and the growth medium used for cultivation. Eighteen strains were able to activate bovine and ovine plasminogen and some of these also activated plasminogen from the horse, rabbit and pig. None activated human plasminogen and one strain (CE127) did not activate plasminogen from any source. Tricine-SDS PAGE and zymographic analysis of culture supernatants showed that bovine plasminogen was activated by four out of six strains at two locations corresponding to 16 kDa and 10 kDa. Following the growth of five strains in the presence of bovine plasminogen, all but strain CE127 bound high levels of plasmin activity. In contrast, following growth in human plasminogen none of the strains exhibited bound plasmin activity although all could bind human plasmin directly. All strains were also able to bind bovine and human plasminogen in such a way as to allow its activation by urokinase. We conclude that *S. dysgalactiae* is capable of activating mammalian plasminogen in a species-specific fashion and that the bacterium is also capable of binding plasmin and plasminogen with an apparent preference for bovine plasmin over human plasmin and/or plasminogen from either species. (C) 1998 Elsevier Science B.V.

L26 ANSWER 43 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:437021 SCISEARCH

THE GENUINE ARTICLE: ZU066

TITLE: The Streptococcus agalactiae hylB gene encoding hyaluronate lyase: completion of the sequence and **expression** analysis

AUTHOR: Gase K; Ozegowski J; Malke H (Reprint)

CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany; Univ Jena, Inst Expt Microbiol, D-07745 Jena, Germany

COUNTRY OF AUTHOR: Germany

SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND EXPRESSION, (29 MAY 1998) Vol. 1398, No. 1, pp. 86-98.  
ISSN: 0167-4781.

PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 43

ENTRY DATE: Entered STN: 1998  
Last Updated on STN: 1998

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We report the cloning, sequencing and **expression** analysis of the Streptococcus agalactiae strain 4755 hylB(4755) allele, the first chromosomally-encoded streptococcal hyaluronate lyase gene to be cloned and sequenced completely. This gene lies in a region homologous to that found in *S. mutans*, between the mutX and rmlB genes, a region involved in the synthesis of the serotype c-specific polysaccharide

antigen of this organism. Sequencing of hylB(4755) revealed a 3216-bp open reading frame that encodes a 121.2-kDa polypeptide possessing a 30-amino acid signal sequence which was theoretically predicted and experimentally confirmed. A recombinant plasmid, pHYB100, containing hylB(4755) together with its promoter and terminator was constructed and used to analyze the expression of the gene in *Escherichia coli*. In Northern hybridization experiments, hylB(4755) was found to be transcribed as 3.3-kb monocistronic mRNA from its own promoter which exhibits an extended, sigma(70)-like 10 consensus sequence. Transcript mapping by primer extension analysis placed the major transcription initiation site leading to the longest transcript 38 bp upstream of the translational initiation codon: ATG. *E. coli* TG1(pHYB100) efficiently synthesized hyaluronan-cleaving enzyme activity at similar to 7000 working units/10<sup>9</sup> cells, with lyase activity detectable in all principle cellular locations. Zymography and Western analysis identified functional activity in TG1(pHYB100) to be associated with similar to 118, 110 and 94-kDa polypeptides, with the two low molecular weight species constituting the major components of the enzyme purified from the culture supernatant fluid of *S. agalactiae* 4755. The 118-kDa form was shown to represent the undegraded mature enzyme, whereas the smaller species are likely to arise from proteolytic cleavage in the N-terminal part of the mature protein. The HylB(4755) protein showed extensive sequence identity to the homologous enzymes from *S. agalactiae* 3502 and *S. pneumoniae* characterized by others but sequence comparisons clearly show that incomplete genes truncated at their 5' ends had been isolated from these two organisms. (C) 1998 Elsevier Science B.V. All rights reserved.

L26 ANSWER 44 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:547160 SCISEARCH

THE GENUINE ARTICLE: XL484

TITLE: The LppC gene of *Streptococcus equisimilis* encodes a lipoprotein that is homologous to the e(P4) outer membrane protein from *Haemophilus influenzae*  
AUTHOR: Gase K (Reprint); Liu G W; Bruckmann A; Steiner K; Ozegowski J; Malke H

CORPORATE SOURCE: UNIV JENA, INST MOL BIOL, D-07745 JENA, GERMANY

COUNTRY OF AUTHOR: GERMANY

SOURCE: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (JUN 1997) Vol. 186, No. 1, pp. 63-73.  
ISSN: 0300-8584.

PUBLISHER: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 39

ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We report the cloning, sequencing, and analysis of a novel chromosomal gene of *Streptococcus equisimilis* strain H46A that codes for a membrane lipoprotein, designated LppC. The lppC gene is located 3' adjacent to, and co-oriented with, the unrelated gapC gene that encodes the previously characterized glyceraldehyde-3-phosphate dehydrogenase. Sequencing of lppC revealed an 855-bp open reading frame that predicted a 32.4-kDa polypeptide possessing a potential lipoprotein signal sequence and modification site (VTGC). Signal sequence processing of LppC synthesized in the homologous host or expressed from plasmid pLPP2 in *Escherichia coli* was sensitive to globomycin, a selective inhibitor of lipoprotein-specific signal peptidase II. Subcellular localization of LppC using polyclonal antibodies raised to the hexahistidyl-tagged protein proved LppC to be tightly associated with the cytoplasmic membrane of *S. equisimilis* and with the outer

membrane of *E. coli* JM109 (pLPP2). Southern, Northern and Western analyses indicated that *Ipl*, was conserved in *S. pyogenes*, and transcribed independently of gap as monocistronic 0.9-kb mRNA from a sigma(70)-like consensus promoter. Database searches found homology of *lppC* to the *hel* gene-encoded outer membrane protein e (P4) from *Haemophilus influenzae* to which it exhibits 58% sequence similarity. However, unlike the *hel* gene, *lppC* was unable to complement *hemA* mutants of *E. coli* for growth on hemin as sole porphyrin source in aerobic conditions. Furthermore, neither the wild type nor an *lppC* insertion mutant of *S. equisimilis* could grow on hemin in iron-limited medium. These results, together with findings indicating that *S. equisimilis* H46A had no absolute requirement for iron, led us to conclude that *lppC*, in contrast to *hel*, is not involved in hemin utilization and has yet to be assigned a function.

L26 ANSWER 45 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1996:43901 SCISEARCH  
 THE GENUINE ARTICLE: TN446  
 TITLE: Cloning and sequencing of the  
**streptokinase** gene from *Streptococcus pyogenes*  
 (CIP 56.57)  
 AUTHOR: Ball M M (Reprint); Puig J; Iborra F  
 CORPORATE SOURCE: UNIV PARIS 11, F-91405 ORSAY, FRANCE  
 COUNTRY OF AUTHOR: FRANCE  
 SOURCE: DNA SEQUENCE, (1995) Vol. 6, No. 1, pp. 33-36.  
 ISSN: 1042-5179.  
 PUBLISHER: HARWOOD ACAD PUBL GMBH, C/O STBS LTD, PO BOX 90, READING,  
 BERKS, ENGLAND RG1 8JL.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: English  
 REFERENCE COUNT: 10  
 ENTRY DATE: Entered STN: 1996  
 Last Updated on STN: 1996

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The **streptokinase** gene of the *Streptococcus pyogenes* strain  
 CIP 56.57 was cloned and sequenced. This sequence coding for a  
 441 amino acid protein is well conserved among streptococcus species:  
 there are two very conserved domains separated by a more variable region.

L26 ANSWER 46 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1995:622543 SCISEARCH  
 THE GENUINE ARTICLE: RU780  
 TITLE: **STREPTOKINASE-MEDIATED PLASMINOGEN ACTIVATION**  
**USING A RECOMBINANT DUAL FUSION PROTEIN**  
**CONSTRUCT - A NOVEL-APPROACH TO STUDY BACTERIAL HOST**  
**PROTEIN INTERACTIONS**  
 AUTHOR: LIZANO S (Reprint); JOHNSTON K H  
 CORPORATE SOURCE: LOUISIANA STATE UNIV, MED CTR, DEPT MICROBIOL IMMUNOL &  
 PARASITOL, NEW ORLEANS, LA 70112  
 COUNTRY OF AUTHOR: USA  
 SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (SEP 1995) Vol. 23,  
 No. 3, pp. 261-280.  
 ISSN: 0167-7012.  
 PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
 NETHERLANDS.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: English  
 REFERENCE COUNT: 59  
 ENTRY DATE: Entered STN: 1995  
 Last Updated on STN: 1995

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB **Streptokinase** (SK), a plasminogen (Pg) activator secreted by groups A, C, and G streptococci, is extensively used as a pharmacological agent in thrombolytic therapy and possibly plays a role in streptococcal invasiveness and disease. SK activates Pg to plasmin (Ps) by forming an activator complex with Pg. However, the molecular basis whereby SK binds and activates Pg remains unclear, in part due to the rapid fragmentation of the SK-Pg complex. This study describes a solid phase approach to study this interaction in which a recombinant SK molecule was constructed with glutathione-S-transferase appended to the NH2 terminus and (Gly)(3)(His)(8) appended to the COOH terminus. This dual fusion protein molecule, immobilized on either Sepharose-S-hexylglutathione or Ni2+ dinitriloacetic acid-Sepharose was then used to study the interaction of SK with Pg. These SK-Pg complexes exhibited amidolytic and proteolytic activity similar to native SK, but the pattern of fragmentation of the SK molecule was dependent upon whether the SK molecule was immobilized either at its NH2- or COOH terminus. This solid phase approach may contribute to a greater understanding of the role of SK in Pg activation by enabling the 'capture' of intact activator complexes under physiological conditions and, in addition, may serve as a useful model to analyze other bacterial-host protein interactions important in the pathogenesis of disease.

L26 ANSWER 47 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:29440 HCAPLUS

DOCUMENT NUMBER: 142:428913

TITLE: An improved process for the simultaneous preparation of extracellular **streptokinase** and its analogues

INVENTOR(S): Dikshit, Kanak Lata; Vyas, Vinay Venkatrao; Mahajan, Ritu; Kaur, Jaodeep; Thapar, Nitika; Phatap, Jitesh; Nihalani, Deepak; Sahni, Girish

PATENT ASSIGNEE(S): Council of Scientific and Industrial Research, India

SOURCE: Indian, 122 pp.

CODEN: INXXAP

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IN 183828	A	20000429	IN 1994-DE1727	19941230
PRIORITY APPLN. INFO.:			IN 1994-DE1727	19941230

AB Extracellular **streptokinase** and its analogs prepared by growing recombinant E.coli in a conventional fermentation medium under stirring and supplemented with aeration, separating the cells from supernatant by known methods followed by recovering and purifying **Streptokinase** and its analogs from supernatant.

L26 ANSWER 48 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:175570 HCAPLUS

DOCUMENT NUMBER: 132:218864

TITLE: **Streptokinase** analogs with low antigenicity for use as thrombolytics

INVENTOR(S): Torrens Madrazo, Isis Del Carmen; Garcia Ojalvo, Ariana; De La Fuente Garcia, Jose De Jesus; Seralena Menendez, Alina

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba

SOURCE: Eur. Pat. Appl., 54 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 985729	A2	20000315	EP 1999-202639	19990813
EP 985729	A3	20000531		
EP 985729	B1	20050427		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9943424	A1	20000309	AU 1999-43424	19990805
AU 769916	B2	20040212		
CA 2277554	C	20041102	CA 1999-2277554	19990806
CA 2277554	AA	20000214		
US 6309873	B1	20011030	US 1999-374038	19990813
AT 294239	E	20050515	AT 1999-202639	19990813
US 6413759	B1	20020702	US 2000-658179	20000908
PRIORITY APPLN. INFO.:			CU 1998-119	A 19980814
			US 1999-374038	A3 19990813

AB **Streptokinase** analogs with antigenic domains modified to minimize antigenicity are described for use in the treatment of clotting-associated disorders. The proteins retain their capacity for plasminogen activator complex formation. The proteins are manufactured by **expression** of the corresponding allele of the *skc2* gene encoding **streptokinase SKC-2** (Heberkinase®). The mols. obtained from present invention can be used in the treatment of disorders as myocardial infarction, pulmonary thromboembolism, surgical complications and other cases of thrombosis.

L26 ANSWER 49 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:579938 HCAPLUS

DOCUMENT NUMBER: 131:183948

TITLE: Manufacture of **streptokinase** for therapeutic use by **expression** of the cloned gene in *Pichia pastoris*

INVENTOR(S): Estrada Garcia, Mario; Rubiera Chaplen, Roger; Perez, Hidalgo; Serrano Doce, Ricardo; Hernandez Marrero, Luciano F.; Rodriguez Collazo, Pedro; Castro Ramirez, Anaisel; Munoz Munoz, Emilio Amable; Bravo Martinez, Walfrido; Campos Somavilla, Magalys; Pedraza Fernandez, Alicia; De la Furente Garcia, Jose de J.; Herrera Martinez, Luis S.

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba

SOURCE: Czech Rep., 18 pp.

CODEN: CZXXED

DOCUMENT TYPE: Patent

LANGUAGE: Czech

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CZ 284692	B6	19990217	CZ 1991-2256	19910719
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A 19900523
			CS 1991-2256	A 19910719
			SU 1991-5001280	A 19910717

AB **Streptokinase** for therapeutic use is manufactured by **expression** of the cloned gene in *Pichia pastoris*. The protein may be secreted into the culture medium or accumulated intracellularly. The gene was cloned from a type C *Streptococcus equisimilis* by PCR. **Expression** of the gene from the AOX1 promoter using *Pichia pastoris* as the host resulted in

the manufacture of an enzyme with a specific activity of 50,000-100,000 fibrin-agarose units/mg protein.

L26 ANSWER 50 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:405093 HCAPLUS

DOCUMENT NUMBER: 131:54027

TITLE: Fibrin-dependent plasminogen activator activity of modified bacterial **streptokinases**

INVENTOR(S): Reed, Guy L.

PATENT ASSIGNEE(S): The President and Fellows of Harvard College, USA

SOURCE: PCT Int. Appl., 73 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9931247	A1	19990624	WO 1998-US26694	19981215
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 9918295	A1	19990705	AU 1999-18295	19981215
US 6210667	B1	20010403	US 1998-211542	19981215
PRIORITY APPLN. INFO.:			US 1997-69497P	P 19971215
			WO 1998-US26694	W 19981215

AB A pharmaceutical composition in a preferred embodiment comprises an isolated bacterial protein **streptokinase** that induces fibrin-dependent plasminogen activation, and methods for dissolving blood clots in a subject using such a composition Two preferred **streptokinase** mutants and truncated derivs. comprising residues 144-293 and residues 60-414 of the *Streptococcus equisimilis* H46A enzyme. Deletion of the first 59 amino acids to product mutant rSK60-414 yielded a protein with a 767-fold decrease in kcat compared to that of rSK1-414, without any significant change in the Km. The N-terminus dets. the clot (fibrin) dependence of plasminogen activation by **streptokinase** and the regulation of plasminogen activation in the presence of fibrin. By virtue of its requirement for fibrin for plasminogen activation in human plasma, and its sparing of fibrinogen during clot dissoln., **streptokinase** deleted of N-terminal amino acid residues is similar to tissue-type plasminogen activator. Embodiments also include a nucleic acid encoding such as a bacterial protein, a nucleic acid encoding such a bacterial protein as a fusion to another protein, an **expression vector** with the nucleic acid, and a host cell transformed with the **expression vector**.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 51 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1997:155090 HCAPLUS

DOCUMENT NUMBER: 126:154444

TITLE: **Streptokinases** analogs resistant to cleavage and inactivation by plasmin

INVENTOR(S): Reed, Guy L.

PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA

SOURCE: PCT Int. Appl., 64 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9641883	A1	19961227	WO 1996-US9640	19960607
W: CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5854049	A	19981229	US 1995-488940	19950609
PRIORITY APPLN. INFO.:			US 1995-488940	A 19950609

AB Streptokinase analogs with altered plasmin-binding features that are resistant to binding by plasmin and subsequent cleavage and inactivation are described for use as thrombolytics with a prolonged serum half-life. Specifically, analogs of the *Streptococcus equisimilis* streptokinase are described. Changes that increase plasminogen resistance include alterations of the plasmin-binding domain and blocking of the N-terminus. Fusion proteins with maltose-binding protein as the N-terminal moiety are prepared and their plasmin resistance and streptokinase activity are described. Similarly, analogs with substitutions of basic amino acids that identify internal plasmin and trypsin cleavage sites were prepared and characterized.

L26 ANSWER 52 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1993:229181 HCAPLUS

DOCUMENT NUMBER: 118:229181

TITLE: Cloning and expression of a gene for streptokinase from a hemolytic *Streptococcus*

INVENTOR(S): Garcia, Mario P. E.; Chaplen, Roger R.; Hidalgo, Aimee P.; Doce, Ricardo S.; Marrero, Luciano F. H.; Collazo, Pedro R.; Ramirez, Anaisel C.; Munoz, Emilio A. M.; Martinez, Walfrido B.; et al.

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba

SOURCE: Can. Pat. Appl., 26 pp.

CODEN: CPXXEB

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2043953	AA	19921206	CA 1991-2043953	19910605
CA 2043953	C	20010612		
HU 62655	A2	19930528	HU 1991-1770	19910527
HU 216073	B	19990428		
SK 279873	B6	19990507	SK 1991-2256	19910719
PRIORITY APPLN. INFO.:			CA 1991-2043953	19910605

AB The gene for streptokinase of a *Streptococcus equisimilis* type C is cloned and expressed in *Escherichia coli* or in yeasts. Expression in yeasts uses the promoter of the AOX1 gene of *Pichia pastoris* to regulate expression. Secretion of the protein was achieved using the cognate signal peptide or one from sucrose invertase. The gene was cloned by PCR amplification using different pairs of primers to clone the gene with or without the signal sequence. Integrating expression vectors for expression of the gene in *Pichia pastoris* with or without secretion of the product were constructed. When the secretory construct was used, streptokinase yields of 1-1.2 g/L were obtained. The protein had the expected biol. activities, and the purified enzyme had a specific activity of 50,000-100,000 units/mg.

L26 ANSWER 53 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:646505 HCAPLUS

DOCUMENT NUMBER: 117:246505

TITLE: **Streptokinase** mutation affecting **skc** expression in homologous and heterologous hosts

AUTHOR(S): Mechold, U.; Muller, J.; Malke, H.

CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Jena, D-6900, Germany

SOURCE: Zentralblatt fuer Bakteriologie, Supplement (1992), 22(New Perspect. Streptococci Streptococcal Infect.), 336-8  
CODEN: ZBASE2; ISSN: 0941-018X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Mutations affecting the level of **streptokinase** gene **skc** expression and/or secretion in homologous and heterologous hosts are phys. characterized. The principal classes of mutations produced included **skc** deletions, IS element insertions, and **skc** duplications. The deletion events, represented by mutations  $\Delta(\text{skc})$ -247 and  $\Delta(\text{skc})$ -305 present in plasmids pMM247 and pMM305, resp., removed a tetrapeptide (F10-L13 or L12-A15) from the hydrophobic core of the **Skc** signal sequence. These mutations, reduced the size, hydrophobicity and predicted alpha-helicity of the central region of the signal sequence. The corresponding plasmids, upon transformation into *E. coli* and *P. mirabilis* L-forms, substantially increased the level of **Skc** expression in either host. In *E. coli*, they also facilitated the export of mature **Skc** into the culture medium. In the gram-pos. hosts, **skc** expression was less dramatically affected; however, the proportion of **Skc** activity found in the culture medium was significantly decreased when compared to the extracellular activity resulting from wild type **skc**. IS1 insertion did not alter the primary structure of the promoter but displaced in upward direction, by 768 bp, a static DNA bending locus having its center some 140 bp upstream of the -35 region in wild type DNA. When studied with plasmid pMM697, this insertion event resulted in severely decreased **Skc** expression in all hosts but, expectedly, did not affect **Skc** secretability. Gene **skc** duplication in the chromosome of the homologous producer strain, *S. equisimilis* H46A, was achieved by a single crossover event between the chromosomes and an integrateable **Skc** plasmid, pSM752, in the region of shared homol. As judged by Southern hybridization, cells transiently supporting the replication of pSM752 gave rise to a stable erythromycin-resistant clone designated H46SM which was plasmid-free and produced **Skc** at levels approx. twice as high as the wild type.

L26 ANSWER 54 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:585755 HCAPLUS

DOCUMENT NUMBER: 117:185755

TITLE: High-level expression of degraded product-free **streptokinase** in *Escherichia coli* by removal of its putative leader sequence. [Erratum to document cited in CA116(13):122160m]

AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung

CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea

SOURCE: Molecules and Cells (1992), 2(1), 119  
CODEN: MOCEEK; ISSN: 1016-8478

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The omission of acknowledgment of a research grant has been corrected The



error was not reflected in the abstract or the index entries.

L26 ANSWER 55 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:441935 HCAPLUS

DOCUMENT NUMBER: 117:41935

TITLE: Cloning and expression of  
streptokinase gene of C-type Streptococcus  
equisimilis

PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB),  
Cuba

SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04030794	A2	19920203	JP 1990-201600	19900731
JP 3127298	B2	20010122		
EP 489201	A1	19920610	EP 1990-201930	19900717
EP 489201	B1	19951115		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 130369	E	19951215	AT 1990-201930	19900717
ES 2081909	T3	19960316	ES 1990-201930	19900717
US 5296366	A	19940322	US 1991-703778	19910522
AU 644657	B2	19931216	AU 1991-78101	19910531
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A 19900523
			SU 1991-5001280	A 19910717

AB The streptokinase (I) gene SKC-2  
,with/without signal sequence, is cloned from C-type S.  
equisimilis ATCC-9542 by the polymerase chain reaction method and  
expressed in Escherichia coli and yeast for com. manufacture  
of I. Genomic DNA of the C-type S. equisimilis was isolated by  
the standard method and amplified with primers derived from the nucleotide  
sequence of SKC to get I gene with/without signal sequence.  
Expression of the I gene in E. coli and Pichia pastoris  
MP-36 mutant were shown. The production of I with these microorganisms were  
≥350 mg/L and ≥1.2 g/L, resp.

L26 ANSWER 56 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:122160 HCAPLUS

DOCUMENT NUMBER: 116:122160

TITLE: High-level expression of degraded  
product-free streptokinase in Escherichia  
coli by removal of its putative leader  
sequence

AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon;  
Chun, Moon Jin; Byun, Si Myung

CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol.,  
Seoul, 130-650, S. Korea

SOURCE: Molecules and Cells (1991), 1(2), 187-92

CODEN: MOCCEK; ISSN: 1016-8478

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The expression vector for streptokinase has been  
constructed from the previously cloned streptokinase  
-coding gene (skc) from Streptococcus equisimilis.  
Because of its deleterious effect on the host cell growth, the leader  
sequence of skc was removed and the leader sequence-deleted  
skc was subcloned into the vector pKK223-3, which contains the  
regulatable tac promoter and rrnB T1T2 transcription terminator, with a

short synthetic oligonucleotide adapter. When this vector, pKS601 having **skc** gene, was **expressed** in *E. coli*, a 47.4-kDa protein was found to be newly accumulated to about 12% of the total cellular proteins, and it was identified as the **streptokinase** by immunoblotting with rabbit anti-**streptokinase** polyclonal serum. The **expressed streptokinase** was free from carboxyl-terminal degraded 44-kDa **streptokinase** and purified to near homogeneity using DEAE-cellulose and Sephadex G-150 columns. Its specific activity was about  $1.3 \times 10^5$  CLN units/mg protein.

L26 ANSWER 57 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:672660 HCAPLUS

DOCUMENT NUMBER: 115:272660

TITLE: **Recombinant** of thrombolytic and fibrinolytic enzymes as inactive dimers linked by sequence recognized by blood coagulation factors

INVENTOR(S): Dawson, Keith Martyn; Hunter, Michael George; Czaplewski, Lloyd George

PATENT ASSIGNEE(S): British Bio-Technology Ltd., UK

SOURCE: PCT Int. Appl., 110 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 4

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9109125	A1	19910627	WO 1990-GB1911	19901207
W: AU, CA, FI, HU, JP, KR, NO, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
CA 2069085	AA	19910608	CA 1990-2069085	19901207
CA 2069085	C	20000201		
CA 2069105	AA	19910608	CA 1990-2069105	19901207
AU 9169540	A1	19910718	AU 1991-69540	19901207
AU 644399	B2	19931209		
ZA 9009853	A	19920826	ZA 1990-9853	19901207
ZA 9009854	A	19920826	ZA 1990-9854	19901207
EP 504241	A1	19920923	EP 1991-900869	19901207
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 155812	E	19970815	AT 1991-900851	19901207
ES 2106073	T3	19971101	ES 1991-900851	19901207
IL 96601	A1	19990509	IL 1990-96601	19901207
JP 05502374	T2	19930428	JP 1991-501314	19911115
JP 2900606	B2	19990602		
US 5434073	A	19950718	US 1992-854596	19920603
FI 9202609	A	19920605	FI 1992-2609	19920605
NO 9202237	A	19920806	NO 1992-2237	19920605
AU 9344976	A1	19931118	AU 1993-44976	19930830
PRIORITY APPLN. INFO.:			GB 1989-27722	A 19891207
			WO 1990-GB1911	A 19901207

AB Fibrinolytic or thrombolytic enzymes are manufactured in a **recombinant** host as inactive fusion proteins containing two or more sequences of the protein linked by a peptide that can be cleaved by a blood-coagulation factor. The construction of **expression** vectors for the manufacture of hirudin or **streptokinase** dimers linked by peptides cleavable by Factor Xa or thrombin for *Escherichia coli* or *Saccharomyces cerevisiae* (with or without product secretion) is described. All the products tested were cleavable by the appropriate factors.

L26 ANSWER 58 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:443480 HCAPLUS

DOCUMENT NUMBER: 115:43480

TITLE: Synthetic genes for **streptokinase** and

streptokinase analogs and their  
expression in *Escherichia coli*

INVENTOR(S): Fujii, Setsuro; Katano, Tamiki; Majima, Eiji; Ogino, Koichi; Ono, Kenji; Sakata, Yasuyo; Uenoyama, Tsutomu

PATENT ASSIGNEE(S): Otsuka Pharmaceutical Factory, Inc., Japan

SOURCE: Eur. Pat. Appl., 76 pp.  
CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 407942	A2	19910116	EP 1990-113099	19900709
EP 407942	A3	19910904		
EP 407942	B1	19951011		
R: AT, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
JP 04011892	A2	19920116	JP 1990-179851	19900706
US 5240845	A	19930831	US 1990-549049	19900706
AU 9058806	A1	19910117	AU 1990-58806	19900709
AU 648029	B2	19940414		
AT 129014	E	19951015	AT 1990-113099	19900709
ES 2078925	T3	19960101	ES 1990-113099	19900709
CA 2020828	AA	19910112	CA 1990-2020828	19900710
PRIORITY APPLN. INFO.:			JP 1989-179432	A 19890711
			JP 1989-307957	A 19891127
			JP 1990-96830	A 19900411

AB Genes encoding streptokinase (I) and its derivs. are synthesized and expressed in a host such as *Escherichia coli* for manufacture of I suitable for clin. application. The DNA encoding natural-type I was synthesized by standard chemical and used for construction of expression plasmid pSKXT, which in turn expressed the I gene using the *E. coli* tac promoter and the blc signal sequence. Efficient expression of the gene in the *E. coli* transformants and purification of the protein product were demonstrated. I analogs with a carboxy-terminal deletions, optionally with internal modifications were also described.

L26 ANSWER 59 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:179754 HCAPLUS

DOCUMENT NUMBER: 114:179754

TITLE: Fusion proteins of streptokinase and human plasminogen

INVENTOR(S): Malke, Horst; Ferretti, Joseph J.

PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Zentralinstitut fuer Mikrobiologie und Experimentelle Therapie, Ger. Dem. Rep.

SOURCE: Ger. (East), 42 pp.  
CODEN: GEXXA8

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 284484	A5	19901114	DD 1989-328631	19890516
PRIORITY APPLN. INFO.:			DD 1989-328631	19890516

AB A hybrid streptokinase is produced in prokaryotic cells. The streptokinase, which displays thrombin selectivity, consists of the N-terminal kringle domains of human plasminogen fused to C-terminal *Streptococcus equisimilis* streptokinase. *Escherichia coli* transformed with plasmids encoding the described fusion

protein fused to the N-terminal hexapeptide of  $\beta$ -galactosidase produced the hybrid **streptokinase** which was purified from cell lysates by immuno-affinity chromatog. and by chromatog. on lysine Sepharose.

L26 ANSWER 60 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:162447 HCAPLUS

DOCUMENT NUMBER: 114:162447

TITLE: **Recombinant manufacture of streptokinase**

INVENTOR(S): Laplace, Frank; Mueller, Joerg; Malke, Horst

PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Patentabteilung, Ger. Dem. Rep.

SOURCE: Ger. (East), 9 pp.

CODEN: GEXXA8

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 282709	A5	19900919	DD 1988-323948	19881227
PRIORITY APPLN. INFO.:			DD 1988-323948	19881227

AB **Streptokinase** from *Streptococcus equisimilis* serotype C is manufactured by **expression** of the *skc* gene in *Escherichia coli*, *Bacillus subtilis*, or other *Streptococcus*. The natural **expression** cassette for the *skc* gene was introduced into a broad host-range vector to give plasmid pMLS10. Transformants of *Streptococcus sanguis* carrying this vector produced 750-1,000 **streptokinase** units/mL in a complex medium after 16 h growth at 36°, at this point the culture reached stationary phase and the enzyme continued to be slowly accumulated.

L26 ANSWER 61 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:96194 HCAPLUS

DOCUMENT NUMBER: 114:96194

TITLE: The leader sequence of **streptokinase** is responsible for its post-translational carboxyl-terminal cleavage

AUTHOR(S): Park, Seung Kook; Lee, Byeong Ryong; Byun, Si Myung

CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea

SOURCE: Biochemical and Biophysical Research Communications (1991), 174(1), 282-6

CODEN: BBRCA9; ISSN: 0006-291X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB When the **streptokinase** gene from *Streptococcus equisimillis* was **expressed** from 2 tac promoter-controlled **expression** vectors, one deleted the putative leader sequence of **streptokinase**. Both normal and degraded **streptokinase** were detected in proteins **expressed** from the leader-encoding vector, but only normal **streptokinase** was detected from the leader-deleted vector. These findings indicate that the characteristic carboxyl-terminal cleavage of **streptokinase** is correlated with its leader sequence and occurs during defective secretion. A homogeneous preparation of **streptokinase** was facilitated by **expression** from this leader-deleted vector.

L26 ANSWER 62 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1989:206803 HCAPLUS

DOCUMENT NUMBER: 110:206803

TITLE: **Streptokinase** mutations relieving

Escherichia coli K-12 (prlA4) of detriments caused by the wild-type **skc** gene

AUTHOR(S): Mueller, Joerg; Reinert, Hilmer; Malke, Horst

CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Therapy, Acad. Sci. G. D. R., Jena, 6900, Ger. Dem. Rep.

SOURCE: Journal of Bacteriology (1989), 171(4), 2202-8  
CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A novel phenotype is described for E. coli K-12 carrying the prlA4 allele determining a membrane component of the protein export mechanism. It is manifest as transformation deficiency for plasmids containing the cloned group C streptococcal **streptokinase** gene, **skc**. **Streptokinase** plasmid mutations relieving the prlA4 strain of this deficiency fell into three classes. Class 1 included **skc::IS5** insertions, with IS5 integrated in a region encoding the **Skc** signal sequence and inactivating **skc**. Class 2 included IS1 insertions leaving **skc** intact but reducing **skc** expression, presumably by altering the function of the **skc** promoter as judged by an insertion site close to the -35 region. Class 3 included **skc** deletions removing the entire signal sequence or a tetrapeptide from its hydrophobic core. The tetrapeptide deletion reduced the size, hydrophobicity, and predicted  $\alpha$ -helicity of the central region of the **Skc** signal sequence but facilitated the export of mature **Skc** in both the wild type and the prlA4 mutant. These findings indicate that the incompatibility between prlA4 and **skc** is related to deleterious effects of the **Skc** signal sequence. The tetrapeptide deletion may function by altering the conformation of the signal sequence so as to render interaction with both the PrlA wild-type protein and the PrlA4 mutant protein less detrimental to the export mechanism. These findings also provide an explanation for the difficulties encountered in cloning **streptokinase** genes in E. coli plasmids and maintaining their structural stability.

L26 ANSWER 63 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1988:487410 HCAPLUS

DOCUMENT NUMBER: 109:87410

TITLE: Methylophilic yeast as vehicles for heterologous gene expression

AUTHOR(S): Stroman, D. W.; Hagenson, M. J.

CORPORATE SOURCE: Phillips Res. Cent., Phillips Pet. Co., OK, USA

SOURCE: DEHEMA Monographien (1987), 105(Physiol. Genet. Modulation Prod. Form.), 141-6  
CODEN: DMDGAG; ISSN: 0070-315X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The methylophilic yeast, Pichia pastoris, has been developed as a superior recombinant DNA (rDNA) production host. The key component in the development of this host was the cloning of the alc. oxidase gene and use of its promoter-regulatory region to control gene expression. Heterologous expression of several foreign genes in this yeast has been studied. The promoter-regulatory region from the alc. oxidase gene permits very high per cell levels of gene expression in an easily regulated manner. These high per cell levels of expression can be combined with high cell d. fermentation technol. to yield very high per L production of rDNA products. This is shown by the high levels of production of **streptokinase** in this yeast.

L26 ANSWER 64 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1987:472094 HCAPLUS

DOCUMENT NUMBER: 107:72094

TITLE: New cloning vectors for Escherichia coli and Bacillus subtilis

INVENTOR(S): Klessen, Christian; Malke, Horst  
 PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Ger. Dem. Rep.  
 SOURCE: Ger. (East), 7 pp.  
 CODEN: GEXXA8  
 DOCUMENT TYPE: Patent  
 LANGUAGE: German  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 231083	A1	19851218	DD 1984-264255	19840619
PRIORITY APPLN. INFO.:			DD 1984-264255	19840619

AB New cloning vectors for *Escherichia coli* and *Bacillus subtilis*, derived from the bifunctional plasmid pGR71 by insertion of a promoter-containing DNA fragment into the unique HindIII site of pGR71 upstream of the chloramphenicol acetyltransferase gene, are described. The new plasmids pSM1711, pSM7711, and pSM7712 contain a *Streptococcus* promoter from plasmids pMF1, pSM10, or pSM7.

L26 ANSWER 65 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:509637 HCAPLUS  
 DOCUMENT NUMBER: 105:109637  
 TITLE: The **streptokinase** gene  
 AUTHOR(S): Malke, H.; Ferretti, J. J.  
 CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Ger. Acad. Sci., Jena, DDR-6900, Ger. Dem. Rep.  
 SOURCE: Folia Haematologica (Leipzig) (1986), 113(1-2), 88-98  
 CODEN: FOHEAW; ISSN: 0323-4347  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB *Streptococcus equisimilis* Gene **skc** for **streptokinase** [9002-01-1] was cloned on the vector L47 and then subcloned into plasmids pACYC184 and pBR322 to form recombinant plasmids pMF2 (10.4 kb) and pMF5 (9.9 kb), resp., for expression in *Escherichia coli*. Plasmid pMF5 contained a 2568-base-pair (bp) insert that included the 1320-bp coding sequence for prestreptokinase. The prestreptokinase comprised 440 amino acid residues, including a 26-amino acid signal peptide. The insert also contained the **skc** upstream regions involved in the regulation of transcription and translation and a 15-bp repeat located 34 bp downstream of the **skc** translation stop signal, which very likely represents the rho-independent transcription terminator. The **skc** gene showed no extended regions homologous to the staphylokinase gene. Heterologous **skc** gene expression was also attained in *S. sanguis* after subcloning of the gene of pMF5 onto plasmid pSM7 to form the bifunctional shuttle plasmid pSM752 (13.3 kb). Plasmid pSM752 was not only functional in *E. coli* and *S. sanguis*, but also in *Bacillus subtilis*. The cloned **streptokinase** expressed in *E. coli*, *S. sanguis*, or *B. subtilis* has the same specificity as that of the donor strain.

L26 ANSWER 66 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:473709 HCAPLUS  
 DOCUMENT NUMBER: 105:73709  
 TITLE: Cloning of streptococcal genes with *Streptococcus-Escherichia coli* shuttle vector pSA3  
 AUTHOR(S): Dao, M. L.; Ferretti, J. J.  
 CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA  
 SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 233-4. Editor(s):

Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi.  
Reedbooks: Bracknell, UK.  
CODEN: 55BSAN

DOCUMENT TYPE: Conference  
LANGUAGE: English

AB A shuttle vector, the chimeric plasmid pSA3, which can replicate in both *E. coli* and *S. sanguis*, was constructed. Chromosomal DNA from *S. mutans* was ligated into this plasmid and cloned in *E. coli*. Of 472 clones tested, 43 clones expressed *S. mutans* surface antigens. A cloned *S. equisimilis* streptokinase [9002-01-1] gene was inserted into plasmid pSA3 and then used to transform *E. coli*, *S. sanguis*, and *S. mutans*, all of which expressed the cloned streptokinase gene.

L26 ANSWER 67 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:473707 HCAPLUS

DOCUMENT NUMBER: 105:73707

TITLE: Cloned streptokinase gene from  
*Streptococcus equisimilis* H46A

AUTHOR(S): Malke, H.; Ferretti, J. J.

CORPORATE SOURCE: Ger. Acad. Sci., Jena, Ger. Dem. Rep.

SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc.  
Lancefield Int. Symp. Streptococci Streptococcal Dis.,  
9th (1985), Meeting Date 1984, 221-2. Editor(s):  
Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi.  
Reedbooks: Bracknell, UK.  
CODEN: 55BSAN

DOCUMENT TYPE: Conference

LANGUAGE: English

AB The streptokinase [9002-01-1] gene *skc* of *S. equisimilis* was cloned in *Escherichia coli* with plasmid pBR322. Expression of gene *skc* was observed with both orientations of the gene, which indicated that its own promoter was present and was functional in *E. coli*. Streptokinase was excreted by the *E. coli* host. The gene contained a 1320-base-pair open reading frame which encodes 440 amino acids, including a signal peptide of 26 amino acids.

L26 ANSWER 68 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1986:455636 HCAPLUS

DOCUMENT NUMBER: 105:55636

TITLE: The streptokinase gene: cloning,  
sequencing and expression in new hosts

AUTHOR(S): Malke, Horst

CORPORATE SOURCE: Zentralinst. Mikrobiol., Dtsch. Akad. Wiss., Jena,  
Ger. Dem. Rep.

SOURCE: Zeitschrift fuer Klinische Medizin (1985) (1986),  
41(7), 502-4

CODEN: ZKMEEF; ISSN: 0233-1608

DOCUMENT TYPE: Journal

LANGUAGE: German

AB The streptokinase (I) [9002-01-1] gene (*skc*) of *Streptococcus equisimilis* H46A was cloned in *Escherichia coli* using vector  $\lambda$ L47. One of the recombinant clones was used to subclone *skc* in *E. coli* plasmid vectors. Plasmids pMF2 (10.4 kilobases, composed of pACYC184 plus a 6.4-kilobase *Eco*RI fragment) and pMF5 (6.9 kilobases, with a 2.5-kilobase fragment in the *Pst*I site of pBR322) determined I formation in *E. coli*; expression of *skc* was independent of its orientation, indicating that the complete gene, together with its control elements, was present. The 2.5-kilobase *Pst*I fragment of pMF5 was isolated and sequenced in the M13 system. Of 2568 base pairs, the largest open reading frame consisted of 1320 base pairs

coding for prestreptokinase, corresponding to I plus its 26-amino acid leader sequence. **Expression of skc** was attained in *S. sanguis* after transformation with the shuttle vector pSM752. In fermentation expts., I production rates of 1500 U/mL were attained, which was below the levels obtained with *S. equisimilis*. Use of pSM752 for similar transformation of *Bacillus subtilis* is briefly discussed.

L26 ANSWER 69 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN  
 ACCESSION NUMBER: 1985:608113 HCAPLUS  
 DOCUMENT NUMBER: 103:208113  
 TITLE: **Streptokinase-coding recombinant vectors**  
 INVENTOR(S): Ferretti, Joseph J.; Malke, Horst  
 PATENT ASSIGNEE(S): Phillips Petroleum Co. , USA  
 SOURCE: Eur. Pat. Appl., 21 pp.  
 CODEN: EPXXDW  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 2  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 151337	A2	19850814	EP 1984-306851	19841008
EP 151337	A3	19861008		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
DD 249037	A1	19870826	DD 1983-255523	19831010
US 4764469	A	19880816	US 1984-585417	19840302
AU 8433859	A1	19850418	AU 1984-33859	19841005
AU 561372	B2	19870507		
ZA 8407873	A	19850529	ZA 1984-7873	19841008
AT 61816	E	19910415	AT 1984-306851	19841008
FI 8403963	A	19850411	FI 1984-3963	19841009
NO 8404039	A	19850411	NO 1984-4039	19841009
DK 8404822	A	19850426	DK 1984-4822	19841009
JP 60237995	A2	19851126	JP 1984-212403	19841009
ES 536623	A1	19870116	ES 1984-536623	19841009
CA 1223223	A1	19870623	CA 1984-464939	19841009
DD 273284	A5	19891108	DD 1984-268254	19841010
US 5066589	A	19911119	US 1988-212254	19880627
US 5187098	A	19930216	US 1992-888420	19920522
PRIORITY APPLN. INFO.:			DD 1983-255523	A 19831010
			US 1984-585417	A 19840302
			EP 1984-306851	A 19841008
			US 1988-212254	A2 19880627
			US 1989-348206	B1 19890509

AB **Recombinant vectors that code for streptokinase** [9002-01-1] are constructed and cloned in *Escherichia coli*. Thus, DNA from *Streptococcus equisimilis* was isolated and digested with the restriction endonuclease *Sau3A*. DNA fragments of between 4-15 kb were cloned into phage  $\lambda$  L47. The ligated phage was infectively added to *E. coli* lawns and **streptokinase-producing clones** were isolated. The DNA from one such clone,  $\lambda$  L47E **skc** was partially digested with *HindIII* and then inserted into the *HindIII* site of plasmid pBR322. The **recombinant** plasmids were used to transform *E. coli* strain HB101. The plasmid isolated from 1 **streptokinase-producing strain**, pMF1, was isolated and a restriction map was prepared. A nucleotide sequence anal. of pMF1 showed that the cloned fragment encoded for **streptokinase** as well as an amino-terminal signal peptide which is bound to **streptokinase** and which is hydrolyzed during a **streptokinase secretion event**.



L26 ANSWER 70 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1985:107414 HCAPLUS

DOCUMENT NUMBER: 102:107414

TITLE: Streptococcus-Escherichia coli shuttle vector pSA3 and its use in the cloning of streptococcal genes

AUTHOR(S): Dao, My Lien; Ferretti, Joseph J.

CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, 73190, USA

SOURCE: Applied and Environmental Microbiology (1985), 49(1), 115-19

CODEN: AEMIDF; ISSN: 0099-2240

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A shuttle vector that can replicate in both Streptococcus and E. coli was constructed by joining the E. coli plasmid pACYC184 (chloramphenicol [56-75-7] and tetracycline [60-54-8] resistance) to the streptococcal plasmid pGB305 (erythromycin [114-07-8] resistance). The resulting chimeric plasmid is designated pSA3 (chloramphenicol, erythromycin, and tetracycline resistance) and had 7 unique restriction sites: EcoRI, EcoRV, BamHI, SalI, XbaI, NruI, and SphI. Mol. cloning into the EcoRI or EcoRV site results in inactivation of chloramphenicol resistance, and cloning into the BamHI, SalI site results in inactivation of tetracycline resistance in E. coli. Plasmid pSA3 was transformed and was stable in S. sanguis and S. mutans in the presence of erythromycin. Plasmid pSA3 was used to construct a library of the S. mutans GS5 genome in E. coli, and expression of surface antigens in this heterologous host was confirmed with S. mutans antiserum. A previously cloned determinant that species streptokinase [9002-01-1] was subcloned into pSA3, and this recombinant plasmid was stable in the presence of a selective pressure and expressed streptokinase activity in E. coli, S. sanguis, and S. mutans.

L26 ANSWER 71 OF 71 LIFESCI COPYRIGHT 2005 CSA on STN

ACCESSION NUMBER: 96:44932 LIFESCI

TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus equisimilis

AUTHOR: Mechold, U.; Cashel, M.; Steiner, K.; Gentry, D.; Malke, H.

CORPORATE SOURCE: Inst. Molecular Biol., Jena Univ., Winzerlaer Str. 10, D-07745 Jena, Germany

SOURCE: J. BACTERIOL., (1996) vol. 178, no. 5, pp. 1404-1411.

ISSN: 0021-9193.

DOCUMENT TYPE: Journal

FILE SEGMENT: J; G

LANGUAGE: English

SUMMARY LANGUAGE: English

AB We examined the functional attributes of a gene encountered by sequencing the streptokinase gene region of Streptococcus equisimilis H46A. This gene, originally called rel, here termed rel sub( )S. equisimilis, is homologous to two related Escherichia coli genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. coli mutants led us to deduce that the highly expressed rel sub( )S. equisimilis gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel sub( )S. equisimilis protein, purified from an E. coli relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolysis reaction similar to that of the SpoT enzyme. This Rel sub( )S. equisimilis protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP

3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel sub( )S. *equisimilis* protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel sub( )S. *equisimilis* gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel sub( )S. *equisimilis* in S. *equisimilis* abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

=> d his

(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

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L1      42118 S STREPTOKINASE?
L2      630 S "SKC" OR "SKC-2"
L3      42539 S L1 OR L2
L4      7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5      4154 S L3 AND L4
L6      1362 S EQUISIMILIS
L7      220 S L5 AND L6
L8      49433 S INCLUSION (W) BOD?
L9      5 S L7 AND L8
L10     1 DUP REM L9 (4 DUPLICATES REMOVED)
L11     106 DUP REM L7 (114 DUPLICATES REMOVED)
L12     5 S LAMNDA
L13     1 S L11 AND INCLUSION
L14     0 S L11 AND AGGREGAT?
L15     0 S L11 AND INSOLUBLE
L16     1 S L11 AND SOLUBL?
L17     71 S L11 AND COLI
        E KUPPUSAMY M/AU
L18     40 S E3
        E ELLA K/AU
        E KHATRI G S/AU
L19     46 S E3
        E LAHIRI S/AU
L20     1660 S E3
        E SRINIVAS V K/AU
L21     28 S E3
L22     1842 S L17 OR L18 OR L19 OR L20 OR L21
L23     71 S L3 AND L22
L24     1 S L8 AND L23
L25     71 S L4 AND L23
L26     71 S L6 AND L25
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=> s l26 and l8

L27 1 L26 AND L8

=> d ibib ab

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L27 ANSWER 1 OF 1  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation  on STN
ACCESSION NUMBER:  2000:96119  BIOSIS
DOCUMENT NUMBER:   PREV200000096119
TITLE:             Two streptokinase genes are expressed
                   with different solubility in Escherichia coli
                   W3110.
AUTHOR(S):         Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.;
                   Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens,
                   Isis
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CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba  
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.  
CODEN: BILED3. ISSN: 0141-5492.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 15 Mar 2000  
Last Updated on STN: 3 Jan 2002

AB The streptokinase (SK) gene from *S. equisimilis* H46A (ATCC 12449) was cloned in *E. coli* W3110 under the control of the tryptophan promoter. The recombinant SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from *S. equisimilis* (ATCC 9542) which had been cloned in *E. coli* W3110 by using similar expression vector and cell growth conditions, and occurred in the form of inclusion bodies.

=> d his

(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

L1 42118 S STREPTOKINASE?  
L2 630 S "SKC" OR "SKC-2"  
L3 42539 S L1 OR L2  
L4 7454183 S CLON? OR EXPRESS? OR RECOMBINANT  
L5 4154 S L3 AND L4  
L6 1362 S EQUISIMILIS  
L7 220 S L5 AND L6  
L8 49433 S INCLUSION (W) BOD?  
L9 5 S L7 AND L8  
L10 1 DUP REM L9 (4 DUPLICATES REMOVED)  
L11 106 DUP REM L7 (114 DUPLICATES REMOVED)  
L12 5 S LAMNDA  
L13 1 S L11 AND INCLUSION  
L14 0 S L11 AND AGGREGAT?  
L15 0 S L11 AND INSOLUBLE  
L16 1 S L11 AND SOLUBL?  
L17 71 S L11 AND COLI  
E KUPPUSAMY M/AU  
L18 40 S E3  
E ELLA K/AU  
E KHATRI G S/AU  
L19 46 S E3  
E LAHIRI S/AU  
L20 1660 S E3  
E SRINIVAS V K/AU  
L21 28 S E3  
L22 1842 S L17 OR L18 OR L19 OR L20 OR L21  
L23 71 S L3 AND L22  
L24 1 S L8 AND L23  
L25 71 S L4 AND L23  
L26 71 S L6 AND L25  
L27 1 S L26 AND L8